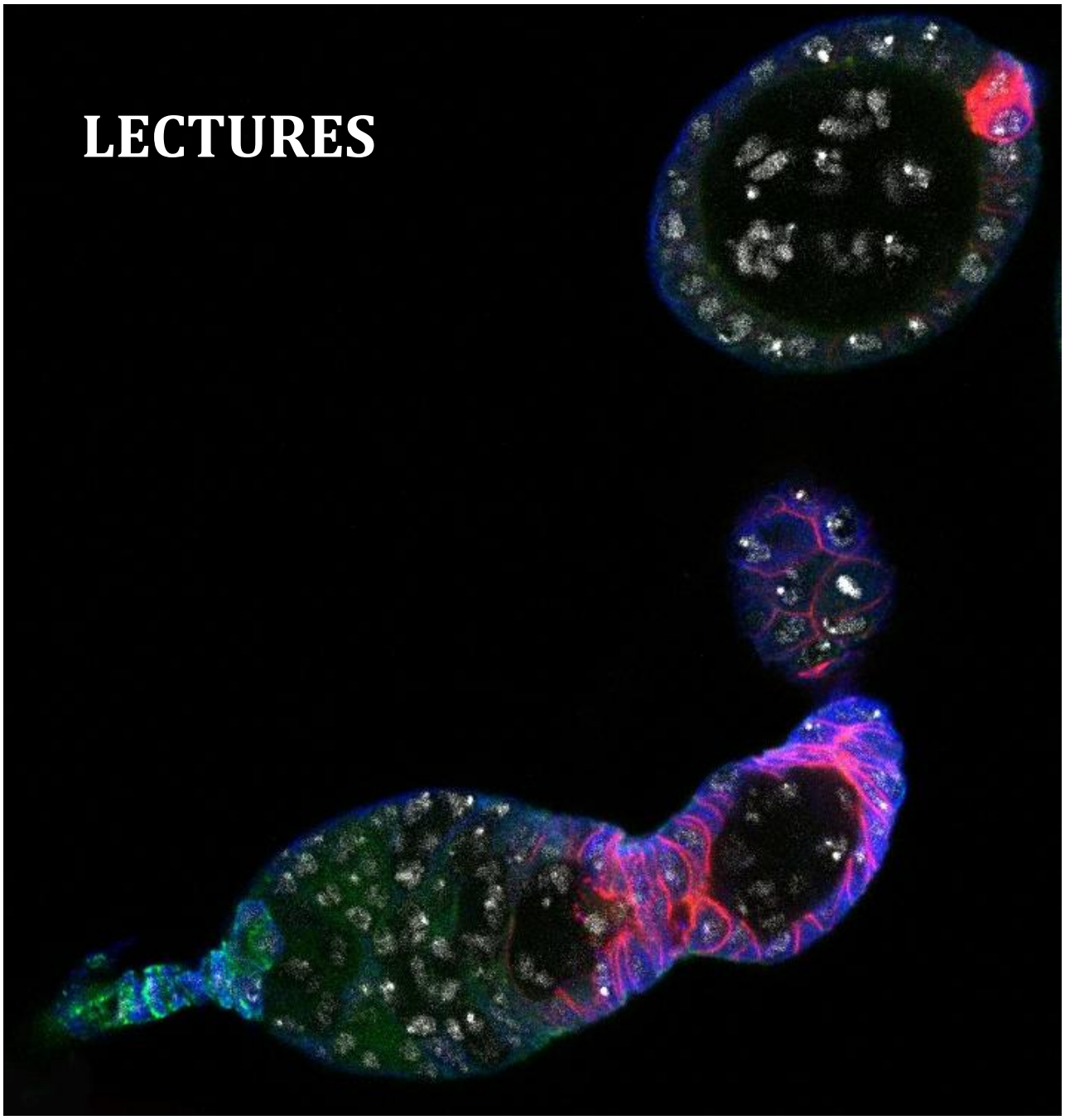
ABSTRACTS

Volume 59

LECTURES



Assembly and transport of RNPs in the *Drosophila* oocyte

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mRNA localization to specific subcellular sites within cells is a powerful and conserved phenomenon that allows precise spatial and temporal control of protein synthesis. *oskar* mRNA localization at the posterior pole of the *Drosophila* oocyte is essential for proper patterning of the embryo. Assembly of transport competent *oskar* mRNPs begins in the nucleus and involves both splicing of the first *oskar* intron and the four core proteins of the Exon Junction Complex (EJC). In the cytoplasm, *oskar* mRNPs associate with motor proteins that transport the mRNA to its destination at the posterior cortex of oocyte. The importance of splicing of intron 1 is due to its requirement for assembly of a posterior targeting element, the Spliced *oskar* Localization Element (SOLE), from a bipartite sequence composed of exonic sequences flanking the intron. This element forms a stem-loop structure that is positioned immediately next to the EJC deposition site and is critical for efficient transport of *oskar* mRNA to the posterior pole. How the EJC dissociates from mRNAs in vivo is unknown. New findings will be presented concerning the function of PYM, a protein shown to bind the EJC and cause its disassembly in cultured cells, in *Drosophila* oogenesis.

Cytosine-5 RNA methylation in normal tissues and diseases

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Cytosine-5 methylation (m^5C) is a widespread modification in both DNA and RNA and the corresponding methyltransferases share many structural features. Whereas the functions of m^5C in DNA have been extensively studied, the cellular and molecular functions of the same modified nucleobase in RNA remain unclear. We found that m^5C is a common post-transcriptional modification in transfer RNA (tRNA) and other non-coding RNA species. RNA-methylation pathways is an important regulator of stem cell differentiation in various tissues, and loss-of-function mutations in the cytosine-5 RNA methylase NSun2 causes neuro-developmental diseases in humans. Depletion of the m^5C modification in tRNAs causes their cleavage by angiogenin and the cleaved tRNA fragments are implicated in the reduction of protein translation rates in response to cellular stress stimuli. Thus, post-transcriptional cytosine-5 methylation is an important and unexpected regulatory pathway to control cellular behavior.

Developmental programs controlling diversification of muscle types and specification of muscle stem cells in *Drosophila*

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In all metazoan organisms, the diversification of cell types involves determination of cell fates and subsequent execution of specific differentiation programmes. During *Drosophila* myogenesis, identity genes specify the fates of founder myoblasts, from which derive all individual larval muscles and stem cell-like Adult Muscle Precursors (AMPs). Here, to understand how cell fate information residing within founders is translated during differentiation, we focus on three identity genes, *eve*, *lb* and *slou* and how they control the size of individual muscles by regulating the number of fusion events. We also use *Drosophila* model to follow specification and *in vivo* behavior of AMP cells.

We report that emerging AMPs display homing behavior, and that muscles act as their niche by protecting dormant AMPs from apoptosis. We also demonstrate that muscles send local inductive dIlp6 signals, which at the end of second larval instar activate proliferation of AMPs. Unexpectedly, genetic rescue experiments reveal that the Insulin pathway acts upstream of Notch, and positively regulates proliferation of AMPs *via* dMyc. Thus we provide evidence for a muscle-specific developmental fusion programs and for muscle-driven Insulin-Notch-Myc cascade setting the activated state of AMPs in *Drosophila*.

Laminin-binding integrins in health and disease

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Our research focuses on the α3β1 and α6β4 integrins, which are receptors for members of the laminin family of basement components. Although the two molecules also associate with the tetraspanin CD151 at the cell surface, it is in particular α3β1 that binds directly and robustly to this protein and it is associated with the actin cytoskeleton in focal adhesions. The α3β1/CD151 ternary complex is also present in the glomerulus of the kidney. We previously generated genetically modified mice lacking α3β1 or CD151 in the glomerulus, and found that they develop progressive renal failure with a decreased glomerular filtration capacity. We have shown that blood pressure is an important determinant of this progressive disease and are now working on deciphering the underlying molecular mechanisms involved. We are also studying the role of the α3β1/CD151 complex in skin cancer and have identified an important function of this complex in the development and progression of skin tumors by controlling the migration of epidermal stem cells from their primary niche in the hair follicle bulge.
In contrast to α3β1, the α6β4 integrin is concentrated in so-called hemidesmosomes (HDs). HDs facilitate firm adhesion of epithelia to the basement membrane. These structures are formed when the integrin α6β4 ligates to laminin-332 in the extracellular matrix and becomes connected to the intermediate filament system by binding to the cytoskeletal linker protein plectin. These two proteins then serve as a scaffold for the recruitment of two other proteins BP180 and BP230 that help to stabilize the HDs. A major focus of our work concerns the mechanisms that regulate the assembly and disassembly of HDs. This is important for understanding how epithelial sheets maintain their structural integrity, how keratinocytes divide and migrate during wound healing and how carcinomas develop and progress.
We have shown that HD dynamics are altered downstream of epidermal growth factor (EGF) receptor activation, following the phosphorylation of integrin β4 residues S1356, S1364 and T1736, which reduces the interaction of β4 with plectin. One of the three major signalling pathways downstream of the EGFR stimulation involves the activation of the conserved MAPK signalling pathway. We have shown that two components of this signal transduction pathway, ERK1/2 and its downstream effector kinase p90RSK1/2, phoshorylate β4 on S1356 and S1364, respectively. T1736 is a substrate for protein kinase D1 *in vitro* and in cells, which requires its translocation to the plasma membrane and subsequent activation. However, evidence that this kinase is also responsible for mediating β4 phosphorylation downstream of EGFR stimulation is still lacking.

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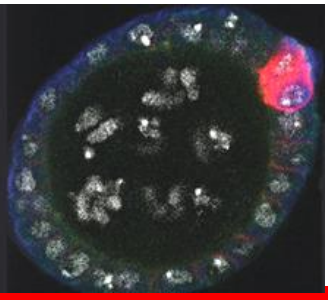

LECTURES

Immunological Mechanisms of chronic inflammation and fibrosis

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Macrophages are found in close proximity with collagen-producing myofibroblasts and play key roles in the mechanisms of wound healing and fibrosis. They produce growth factors and pro-fibrotic mediators that directly activate fibroblasts, including transforming growth factor beta, insulin-like growth factor, vascular endothelial growth factor, and platelet-derived growth factor. They also regulate extracellular matrix turnover by influencing the balance of various matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases. Macrophages also regulate fibrogenesis by secreting chemokines that recruit fibroblasts and other inflammatory cells and by producing various inflammatory and anti-inflammatory cytokines. With their potential to act in both a pro- and anti-fibrotic capacity at distinct stages of the wound healing response, macrophages and the factors they express are integrated into all stages of the fibrotic process. These various and sometimes opposing functions are performed by distinct macrophage subpopulations, the identification of which is a growing focus of fibrosis research. Although collagen-secreting myofibroblasts once were thought of as the master ‘‘mediators” of fibrosis, in this presentation I will illustrate how macrophages function as the master ‘‘regulators’’ of fibrosis.

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LECTURES

bHLH-Orange proteins and Notch signalling in neural stem cell maintenance in Drosophila

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The Notch pathway, the foremost cell-contact mediated signalling pathway in animals, is notorious for its pleiotropy, regulating a large number of biological processes in many tissues and at many times. A common denominator of almost all Notch dependent processes is the induction of one or more bHLH-O genes. bHLH-O genes, which co-evolved with Notch coincidently with the origin of animal multicellularity, constitute one of the classes of basic-helix-loop-helix transcription factors and are characterized by an additional α-helical domain, known as the Orange domain. bHLH-O proteins in turn are divided into the Hes and Hey subclasses. Both of these subclasses seem to act as repressors and collectively are involved in a multitude of developmental cell fate decisions. The *Drosophila* genome harbours 12 *Hes*-type genes and a single *Hey*. We have been studying several bHLH-O genes in the fly in order to gain insight into their function as well as their connection with the Notch pathway.

In this presentation the focus will be the role of bHLH-O factors in the regulation of neural stem cell self-renewal and differentiation. *Drosophila* neuroblasts are asymmetrically dividing stem cells born early in embryogenesis and responsible for the production of almost all central nervous system cells. A typical neuroblast (NB) divides to self-renew and produce a more differentiated ganglion mother cell (GMC). The latter divides only once to produce two neurons or glia cells. When the larva hatches at the end of embryogenesis, the NBs enter quiescence, but get reactivated about a day later and continue proliferating to add new neurons/ glia to the CNS as the larva feeds and grows. NB proliferation terminates, either by a terminal symmetric 2xGMC division or by apoptosis, well into pupation, after all cells needed fore the adult CNS have been produced. At each NB division, the newly born GMC sends a signal back to its sister NB via the Notch pathway and this in turn activates the expression of *E(spl)mγ* and *E(spl)m8*, two of the 12 fly *Hes* genes. Notch also stimulates the expression of a third *Hes* gene, *deadpan* (*dpn*). Via genetic mosaic analysis we showed that the *E(spl)* genes and *dpn* are redundantly required for NB self-renewal, as triply mutant NBs prematurely undergo a symmetric division and terminate before pupation.

Downregulation of Notch in the NBs eliminates *E(spl)mγ*, but only shows a mild underproliferation phenotype. This seems to depend on the continued expression (at lower levels) of *E(spl)m8* and *dpn*. However over-activation of Notch results in extensive NB hyperplasia at the expense of differentiating GMCs, neurons and glia cells. To gain more insight on this pre-tumorous phenotype and to identify novel target genes activated by Notch in the NBs, we performed microarray transcriptome analysis of these hyperplastic CNSs compared to wt controls. We focussed on a set of upregulated genes, which at the same time were ChIP positive for Su(H), the transcription factor that tethers activated Notch (Nicd, i.e. the Notch intracellular domain) to chromatin. We compared this gene cohort of over a hundred genes with several other published data sets regarding either Notch target genes in other contexts or neuroblast-specific genes (in the wt). Interestingly, although a large number of our target genes were bona-fide neuroblast genes, there was only moderate overlap with Notch target genes in cultured cells or the wing epithelium. This reveals a remarkable context specificity in the response to Notch signalling. We are currently validating a number of predicted enhancer elements from these target genes – most of them are highly NB specific. We are also investigating the participation of the top upregulated transcription factors, besides E(spl) and Dpn, in the Notch induced over-proliferation phenotype.

A second asymmetric cell division takes place in the developing CNS. Each GMC gives rise to two different neurons/glia and this cell-fate difference depends on Notch signalling being selectively received by one of the two daughter cells. In this context *E(spl)mγ* is not detectably expressed, but another bHLH-O gene, *Hey*, is turned on in response to Notch. We are comparing enhancer elements of the *Hey* gene with our NB-specific enhancers from the aforementioned microarray analysis to understand the enhancer grammar that dictates cell context specificity in the response to a signalling pathway. We are also generating *Hey* mutations and overexpression costructs to address the role of this heretofore little-studied gene in cell fate decisions.

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EEBMB
LECTURES

How to make a spindle: a new role for the chromatin remodeling factor CHD4

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The mitotic spindle segregates duplicated chromosomes accurately into daughter cells. The spindle assembly checkpoint delays cell division until correct chromosome segregation can be guaranteed, thus maintaining genome stability. When the fidelity of this process is compromised however, abnormal numbers of chromosomes can be distributed to the daughter cells (aneuploidy), which is often associated with cancer.

For many years it was thought that chromosomes were passengers in this process, but it is now clear that they play a major role in spindle assembly. A key component in this regulation is the small GTPase Ran. Production of the GTP bound form of Ran around chromosomes induces spindle assembly, by activating nuclear localization signal – (NLS) containing proteins. In the past we have identified HURP (Hepatoma Up-Regulated Protein) as one of Ran-GTP regulated proteins, which disturbs chromosome segregation, ignoring the spindle checkpoint (1,2,3). HURP's distribution on microtubules (MTs) is regulated by the mitotic kinase Aurora A (3). Moreover, HURP is over-expressed in cancers, suggesting a role in tumorigenesis,

In an effort to identify new Ran-regulated spindle associated factors, we found an unexpected protein: the CHD4 (**C**hromodomain-**h**elicase-**D**NA-**b**inding protein **4)**, a chromatin-remodeling ATPase and a catalytic subunit of the NuRD (nucleosome-remodeling deacetylase) complex. We identified CHD4 as a RanGTP-dependent MT-associated protein (MAP) that localizes to spindle MTs and is essential for spindle assembly. MT binding of CHD4 is via the N-terminal region of the protein that contains an NLS and chromatin-binding domains. During interphase CHD4 is nuclear and separate from MTs. In mitosis, CHD4 mostly dissociates from chromatin and partially localizes to spindle MTs. CHD4 silencing by RNAi in both HeLa and *Drosophila* S2 cells induces defects in spindle assembly and chromosome alignment in early mitosis, leading to chromosome missegregation. Further analysis in *Xenopus* egg extracts and in HeLa cells reveals that CHD4 is a RanGTP-dependent MT stabilizer. Importantly, this function of CHD4 is independent of its chromatin remodeling function (4). Therefore we uncovered a new role for CHD4 as a MAP in spindle assembly that is essential for chromosome segregation. Interestingly, CHD4 plays an important role in DNA-damage repair. The two functions of CHD4 are distinct, but both critical in maintaining genome integrity.

Whether HURP- and CHD4-containing complexes regulate spindle assembly cooperatively or distinctly will be discussed.

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2. HURP wraps microtubule ends with an additional tubulin sheet that has a novel conformation of tubulin. Koffa MD, Santarella RA, Tittmann P, Gross H, Hoenger A. J Mol Biol. 2007 365(5):1587-1595.

3. Kesisova IA, Nakos KC, Tsolou A, Angelis D, Lewis J, Chatzaki A, Agianian B, Giannis A, Koffa MD. Tripolin A, a novel small-molecule inhibitor of aurora A kinase, reveals new regulation of HURP's distribution on microtubules. PLoS One. 2013;8(3):e58485.

4.Yokoyama H, Nakos K, Santarella-Mellwig R, Rybina S, Krijgsveld J, Koffa MD, Mattaj IW. CHD4 is a RanGTP-dependent MAP that stabilizes microtubules and regulates bipolar spindle formation. Current Biology, in press

RNA-binding proteins and Neurodegeneration

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Alterations in the functions of RNA-binding proteins can contribute to neurodegenerative diseases. The family of Elavl/Hu proteins (Embryonic Lethal Abnormal Vision/Paraneoplastic Hu Antigens) appear as relevant to neuronal responses due to the neuron-restricted expression of 3 of its members (nElavls). The fourth and ubiquitously expressed member – Elavl1/HuR – has been considered as "silent" and "overlapping" to nElavls and as such has not been considered as a determinant of neuronal physiology and/or pathology. However, the potency of HuR in regulating ontogenic, inflammatory and stress related post-transcriptional programs have been proven for many tissues. Using tissue restricted transgenesis we identify HuR's involvement in neuroprotection. Furthermore the application of a "ribonomic" platform for the holistic detection of HuR:RNA interactions revealed HuR's potency in the post-transcriptional regulation of RNAs acting against neurodegeneration which can be exploited further as therapeutic targets.

From Genome Integrity to Genome Plasticity: Views into Live Cells

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All cells must ensure that their genomic information is preserved, accurately replicated and passed down unaltered to the daughter cells at every cell division. Multi-subunit protein complexes associate with chromatin to control DNA replication and orchestrate cellular responses to DNA damage. Defects in these control pathways lead to genomic instability and have been directly linked to tumorigenesis. We use functional imaging to study complexes maintaining genome integrity within the context of living cells. We show that the MCM replicative helicase loads onto chromatin to license replication in multiple distinct steps: transient recruitment onto chromatin in mitosis is converted to stable loading during the G1 phase, followed by a wave of additional loading prior to S-phase onset and dissociation from chromatin during S-phase. Our data show that the majority of chromatin is licensed in late G1 phase and suggest that precautious entry into S-phase would lead to under-licensing and DNA replication stress. MCM loading after S-phase entry leads to rereplication of the genome. We have developed a stochastic hybrid model of DNA re-replication which permits genome-wide analysis of re-replication kinetics. The model has been used to visualize how different regions along the genome respond to over-replication in different cells in a population and to capture and analyze cell-to-cell heterogeneity inherent in the re-replication process. Analysis of re-replication dynamics at the single-cell level provide evidence for a high degree of genome plasticity. We propose that DNA rereplication may be exploited by cells to permit robustness under evolutionary pressure.

Regulation of cellular homeostasis by the cylindromatosis tumor suppressor

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Cyld is a tumor suppressor gene that was originally identified as the defective genetic factor that underlies the development of skin adnexal tumors called cylindromas and trichoepitheliomas. During the past decade *Cyld* downregulation or inactivation has been associated with many different types of malignancies, including B cell lymphomas, lung, liver and colon carcinomas. *Cyld* encodes a deubiquitinating enzyme (CYLD) that hydrolyzes selectively K63-linked and M1-linked polyubiquitin chains. It can regulate cellular homeostasis by modulating the activity of various growth, survival and death pathways that include MAPK, NF-kappaB and necroptosis associated pathways. In order to characterize the function of CYLD in vivo, several mouse models of tissue-specific CYLD inactivation have been generated. These models identified critical roles for CYLD in the regulation of distinct molecular homeostatic mechanisms that are essential for the prevention of pathologies. The CYLD-dependent molecular pathways that underlie the development of T cell, hepatocellular and skin pathologies will be presented and analyzed.

Signaling and trafficking of TGFβ family ligands/receptors.

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Spatio-temporal regulation of signaling components plays an important role in the signaling outcome. Indeed, every signaling receptor undergoes internalization by one of at least five different entry routes of endocytosis, clathrin-mediated endocytosis being the most known. Thus, the strength and duration of the signals emanating from ligand/receptor complexes is influenced by alternative sorting events such as recycling and transport to the lysosomal compartment for ligand/receptor degradation, but also by recruiting downstream effectors of signaling complexes.

The TGFβ family ligands signal through heteromeric complexes of transmembrane type I and type II serine threonine kinase receptors, which phosphorylate R-SMAD proteins and oligomerize with SMAD4 being translocated to the nucleus, where they regulate transcription using a large network of interactions with transcription factors, co-activators and co-repressors. Members of this family play critical roles during development being key regulators of stem cells. Indeed, Activin A can maintain pluripotency of hESCs in serum- and feeder-free conditions by enhancing *NANOG* gene transcription via phosphorylation of SMAD2/3. NANOG interacts directly with the SMAD2/3 proteins to modulate (decrease) their transcriptional activity.

We have defined the internalization routes of Activin A receptors (ActRIIB and ALK4) in the presence or absence of Activin A in endothelial cells using confocal microscopy, functional assays and route modulation. Thus, the background methodology is established to determine ligand/receptor internalization routes in hESCs during pluripotency and differentiation.

Small Leucine Rich Proteoglycans-key mediators of bone cell functions

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The class of small leucine-rich proteoglycans (SLRPs) is a family of homologous proteoglycans harboring relatively small (36-42 kDa) protein cores compared with the larger cartilage and mesenchymal proteoglycans. SLRPs have been localized to most skeletal regions, with specific roles designated during all phases of bone formation, including periods relating to cell proliferation, organic matrix deposition, remodeling, and mineral deposition. This is mediated by key signaling pathways regulating the osteogenic program, including the activities of TGF-β, bone morphogenetic protein, Wnt, and NF-κB, which influence both the number of available osteogenic precursors and their subsequent development, differentiation, and function. On the other hand, SLRP depletion is correlated with degenerative diseases such as osteoporosis and ectopic bone formation; whereas aberrant SLRP expression has been associated with the malignant behaviour of transformed cell of the osteoblast lineage. The roles of SLRPs in bone physiology and pathology will be discussed.

Hypoxia-Inducible Factors: guardians of metabolic homeostasis and anti-cancer therapy targets

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Hypoxia-inducible factors (HIFs) are transcriptional activators essential for adaptation to low oxygen conditions. They mediate changes in O_2 delivery and consumption by regulating erythropoiesis, angiogenesis and reprogramming of cellular metabolism under both physiological and pathological conditions. Cancer cells take advantage of HIFs in order to proliferate in the hypoxic microenvironment of solid tumors. HIFs are, therefore, considered promising targets of anticancer therapy.

Many HIF-1 gene targets mediate the shift from oxidative (aerobic) to glycolytic (anaerobic) metabolism under reduced but also under normal O_2 levels, the latter being a unique characteristic of cancer cells termed the Warburg effect. To understand further the role of HIFs in metabolic homeostasis, we have recently examined the effect of hypoxia on lipid accumulation in human cancer cell cultures. We showed that hypoxia stimulates triglyceride formation and identified lipin 1, a phosphatidate phosphatase isoform that catalyzes the penultimate step in triglyceride synthesis, as a direct transcriptional target of HIF-1 (1). HIF-1-dependent up-regulation of lipid formation may serve to safely store toxic free fatty acids that accumulate under hypoxia due to impaired oxidative degradation.

The activation of HIFs in cancer cells is often mediated by oncogenic signaling pathways in an oxygen-independent manner. We have indeed identified two kinases that phosphorylate and regulate the activity of HIF-1α. ERK1/2 modifies the C-terminal domain of HIF-1α and stimulates its transcriptional activity by blocking its CRM1-dependent nuclear export (2), inhibiting its interaction with non-nuclear proteins or promoting its binding to chromatin. On the other hand, CK1δ modifies the PAS-B domain of HIF-1α and impairs its association with ARNT, thereby inhibiting the formation of an active nuclear HIF-1 heterodimer (3). Our recent microscopical and live-cell imaging studies confirm the operation of these mechanisms in vivo and suggest that they can be targeted as an effective way to control cell viability under hypoxia.

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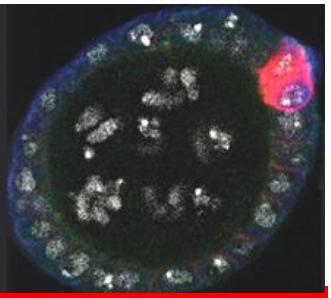

Serglycin: at the crossroad of inflammation and cancer

Theocharis Achilleas
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Serglycin has been initially characterized as an intracellular proteoglycan expressed by hematopoietic cells. It is a dominant proteoglycan in immune cells with major impact on their biology. All inflammatory cells highly synthesize serglycin and store it in granules where interact with numerous inflammatory mediators such as proteases, chemokines, cytokines and growth factors. Serglycin is implicated in their storage into the granules, protection, since they are secreted as complexes and delivery to their targets after secretion. Numerous studies the last decade have demonstrated that serglycin is synthesized by various non-hematopoietic cell types. Recent studies reveal emerging roles for serglycin in tumorigenesis. It has been shown that serglycin is highly expressed by tumor cells and may benefit tumor cells in multiple ways. Serglycin may act as a modulator of immune system in tumor microenvironment and enrich tumor cells with resistance to various therapeutic agents. Serglycin promotes the aggressive phenotype of tumor cells and augments the invasion and metastasis with yet unknown molecular mechanisms. Apart from its direct beneficial role to tumor cells, serglycin may promote the inflammatory process in the tumor cell microenvironment thus enhancing tumor development. Serglycin may serve as molecular partner for proteolytic enzymes, chemokines, cytokines and growth factors protecting and accompanying them to target sites creating chemotactic gradients and enhancing their activities.

Redox regulation of mitochondrial protein import

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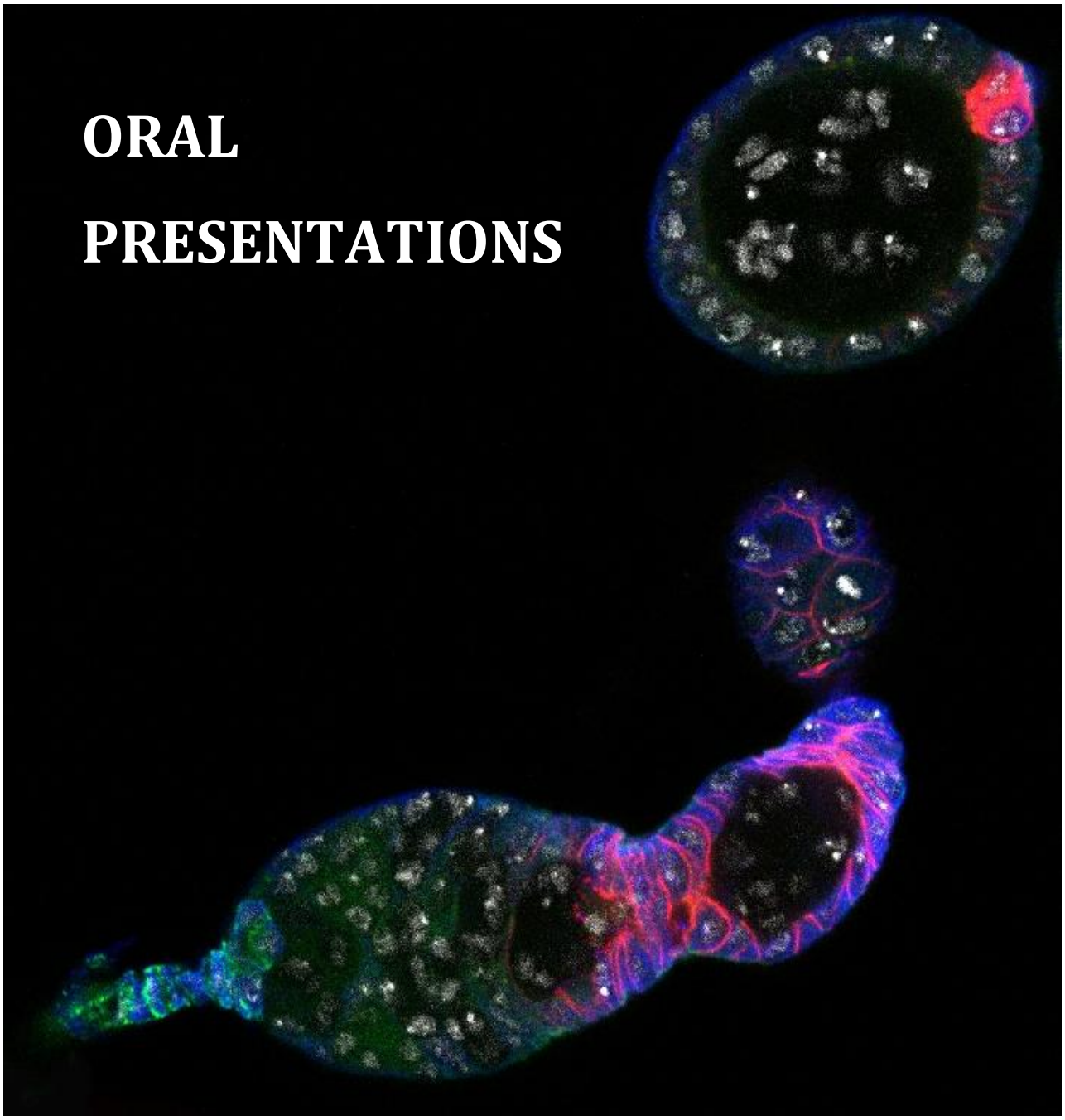
Protein import is the fundamental process underlying mitochondria biogenesis as more than 98% of all mitochondrial proteins are imported from the cytosol. There are distinct protein translocatio machineries (translocons) in the two mitochondrial membranes that have been characterised extensively. The targeting process for several small proteins of the intermembrane space is unique as it is the only one of all mitochondria import pathways that is coupled to a chemical modification of the protein i.e. the formation of internal disulphide bonds. This process of oxidative folding traps the protein in the intermembrane space and is facilitated by two key proteins, the disulphide exchange factor Mia40 and the disulphide donor Erv1. We show that the process is guided by a novel targeting signal called ITS (Import in the Intermembrane Space) that has different chemical properties from the well characterised MTS (Matrix Targeting signal).

We determined that the fundamental role of Mia40 is to chaperone the localised folding of the ITS within the precursor in a process ensured primarily by hydrophobic interactions. Erv1 then recycles Mia40 to its oxidised and functional state by molecular mimicry of the substrate.

Recent studies have reported an expanding role of Mia40 as the major import receptor for proteins of the intermembrane space. Novel aspects of the redox regulation of this pathway and how it relates to mitochondria biogenesis and cell physiology will be discussed.

ORAL PRESENTATIONS



O1

miR-21 is regulated by oncogenes, histone modifiers and controls ITGB4 expression, a novel miR-21 target gene for cell invasion and cancer metastasis

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Alterations of microRNA expression in tumor cells can active the invasion–metastasis cascade, including the Epithelial-Mesenchymal transition (EMT). MiR-21 is considered an oncomir, indeed unlike others miRNAs is frequently upregulated in the majority of solid tumors. We investigated the genetic and epigenetic regulatory pathways that may account for miR-21 over expression and looked for novel miR-21 target genes that mediate regulation of cell invasion and metastasis. For this purpose, miR-21 expression was quantified in a panel of colorectal cancer cell lines and clinical specimens. High expression was found in cell lines with EMT properties and in the vast majority of human tumor specimens. We have demonstrated the occupancy of miR-21 gene promoter by AP-1 and ETS1 transcription factors, and, for the first time, the pattern of histone post-translational modifications necessary for miR-21 over expression in a cell-specific manner. It has been also shown that Integrin-β4 (ITGβ4), exclusively expressed in polarized epithelial cells, is a new miR-21 target gene and the regulates EMT. MiR-21-dependent change of ITGβ4 expression significantly affects cell migration properties of colon cancer cells. Moreover, *ITGβ4* and *PDCD4* expression analysis shows that the combination of high miR-21 with low *ITGβ4* and *PDCD4* expression can predict presence of metastasis (AUC=0,8667; P<0.01). In conclusion, miR-21 is a key player in oncogenic EMT. MiR-21 over expression is controlled by the cooperation of genetic and epigenetic alterations, and relative expression of miR-21 and of its target genes ITGB4 and PDCD4 could be exploited as a prognostic tool for CRC metastasis.

O2

miR-29a-3p and poly(A)-specific ribonuclease expression: a dynamic relation

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MicroRNAs (miRNAs) are small RNAs that mediate the repression of mRNA expression. miRNAs are mainly transcribed by RNA polymerase II as long, capped and polyadenylated precursors, which are further processed to mature miRNAs. They may be expressed in a tissue-specific manner, and are involved in the regulation of almost every cellular process. Aberrant miRNA expression is a hallmark of cancer, however the regulation of miRNA expression remains largely unknown. Recently, factors involved in mRNA degradation have been linked to miRNA biogenesis. Herein, we examine the role of poly(A)-specific ribonuclease, PARN, in the expression of miRNAs. We silenced PARN in NCI-H520 cells of non-small-cell lung cancer (NSCLC) origin, using shRNAs. MiRNA microarrays and qRT-PCR revealed several miRNAs with altered expression. To predict potential targets of the deregulated miRNAs, we used TargetScan, PITA, miRanda and HOCTAR algorithms. Surprisingly, among the upregulated miRNAs, miR-29a-3p may target PARN mRNA. Upon overexpression of miR-29a-3p in NCI-H520 cells, both PARN mRNA and protein levels are reduced. RNA-immunoprecipitation experiments with PARN antibody reveal that PARN associates with miR-29a-3p and its own mRNA, and this association is more abundant in the nuclear than in the cytoplasmic fraction. In NSCLC, miR-29a is known to be downregulated, while recent observations from our lab suggest that PARN shows increased expression. Taken together, our results may suggest a dynamic relation between miR-29a and PARN in lung cancer: miR-29a-3p regulates PARN expression, while PARN is involved in miRNA biogenesis and the regulation of its own levels.

O 3

Spliceosome-mediated decay (SMD) regulates expression of nonintronic genes in budding yeast

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We uncovered a novel role for the spliceosome in regulating mRNA expression levels that involves splicing coupled to RNA decay, which we refer to as spliceosome-mediated decay (SMD). Our transcriptome-wide studies identified numerous transcripts that are not known to have introns but are spliced by the spliceosome at canonical splice sites in Saccharomyces cerevisiae. Products of SMD are primarily degraded by the nuclear RNA surveillance machinery. We demonstrate that SMD can significantly down-regulate mRNA levels; splicing at canonical splice sites in the bromodomain factor 2 (BDF2) transcript reduced transcript levels roughly threefold by generating unstable products that are rapidly degraded by the nuclear surveillance machinery. Regulation of BDF2 mRNA levels by SMD requires Bdf1, a functionally redundant Bdf2 paralog that plays a role in recruiting the spliceosome to the BDF2 mRNA. Interestingly, mutating BDF2 59 splice site and branch point consensus sequences partially suppresses the bdf1D temperature-sensitive phenotype, suggesting that maintaining proper levels of Bdf2 via SMD is biologically important. We propose that the spliceosome can also repress protein-coding gene expression by promoting nuclear turnover of spliced RNA products and provide an insight for coordinated regulation of Bdf1 and Bdf2 levels in the cell.

Reference:

*Volanakis A, Passoni M, Hector RD, Shah S, Kilchert C, Granneman S, Vasiljeva L. 2013. Spliceosome-mediated decay (SMD) regulates expression of nonintronic genes in budding yeast. Genes & development **27**: 2025-2038.*

O 4

Studies on human pnldc1: a parn paralog encoding a novel DEDD deadenylase

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Surveillance and mRNA turnover during translation in human depends on complex degradation pathways mediated by several ribonucleases, which in many cases involve poly(A) tail shortening by human poly(A) specific ribonuclease (hPARN).However, a *parn* paralog termed *pnldc1* of unknown function exists in the human genome, exhibiting low homology (23%) to *hparn* and raising questions on whether this gene also encodes a ribonuclease that is involved on specific deadenylase activities under specific conditions. Surprisingly,an attempt to deplete *parn* expression with siRNAs, did not affected *pnldc1* expression, an observation indicating the functional individuality of both genes. Moreover, *pnldc1* expression was detected only in the presence of demethylating agents like 5-Aza-2'deoxycytidine. Cloning and expression of the recombinant PNLDC1 allowed the intracellular localization of the gene product outside the nucleus and in the ER, in contrast to what has been reported for PARN.Finally, studies in mouse embryos showed that *pnldc1* expression could be detected only in testis and placental stem cells indicating a possible role during development. Biochemical analysis of recombinant hPNLDC1 showed that is indeed a poly(A) specific ribonuclease which can efficiently degrade uncapped mRNAs and has similar biochemical profile to hPARN. Based on the available crystal structure of a truncated hPARN form, we performed *in silico* analyses through homology modeling and molecular dynamic simulations. Our results verified that humanPNLDC1is a *bona fide* DEDD deadenylase and a new member of the mRNA surveillance and turnover repertoire, exhibiting epigenetic regulation, and specific localization and expression with elusive so far physiological role.

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O 5 Thexv f;eree first complete transcriptome study of a female and a male transmitted mitochondrial genome of a species with Doubly Uniparental Inheritance (DUI) of mtDNA

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Many bivalve mollusks carry two mitochondrial genomes in stable coexistence, one transmitted through the eggs (the F type) and one through the sperm (the M type). The phenomenon, known as Doubly Uniparental Inheritance (DUI), older than 200 MY, violates the rule of maternal inheritance in metazoans and is the best known case of obligatory biparental mtDNA inheritance. The M genome is the only known animal mtDNA that is exclusively paternally inherited.

We have studied the 3' and 5' ends of mRNA, rRNA and tRNA transcripts of F and M genomes of *Mytilus galloprovincialis*, the most extensively studied species with DUI. The primary transcript is cleaved into ten mRNA transcripts, eight of which are monocistronic, one is tricistronic and one is most likely, but not certainly, bicistronic. Cleavage is mediated either by the excision of a tRNA or in some cases, by the presence of a stem-loop structure. The identification of a tricistronic transcript is a novel finding for metazoan mtDNA.

We also found polyadenylated and non-adenylated transcripts for both rRNAs and tRNAs, with heterogenous 3' ends. The l-rRNA 3'end was found 48 nucleotides upstream from the one assigned by previous annotation, which makes the adjacent main control region (CR) correspondingly longer. We observed polyadenylated tRNA transcripts carrying the CCA trinucleotide, mRNA, s-rRNA and l-rRNA trancripts with truncated 3' end and polyadenylated RNA remnants carrying the sequences of the control region, all of which strongly suggest RNA degradation activity and thus the presence of degradosomes in *Mytilus* mitochondria.

O 6 RNase κ-02: A widely expressed human endoribonuclease occurring from a subtle alternative splicing event

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Human RNase κ-02 is a novel protein isoform, emerging from a subtle alternative splicing event. The RNase κ-02 mRNA lacks four consecutive bases and encodes the synthesis of a 134 amino-acids protein. The molecular cloning of this novel isoform is very difficult by means of conventional RT-PCR procedures, due to its slight sequence difference from the RNase κ-01 cDNA. For this reason, the RNase κ-02 cDNA cloning was achieved by excluding the RNase κ-01 cDNA from a HEK-293 cDNA pool by combining a modified RT-PCR hybrid selection strategy followed by complete digestion using the appropriate restriction enzyme. Based on the selective digestion of the κ-01 cDNA, we proceeded to the development of a comparative quantitative expression analysis of the two transcripts in several human cell lines by Real-Time PCR. Compared to the already identified RNase κ-01 isoform (98 a.a.), the carboxy-terminal region of RNase κ-02 (a.a. 63-134) is absolutely identical to that of RNase κ-01 (a.a. 27-98), whereas the 1-62 amino-terminal portion bears no similarity. In order to characterize the RNase κ-02 protein, the yeast *P. pastoris* expression system has been employed and the ribonucleolytic properties of the purified recombinant enzyme have been investigated by using a series of labeled RNA substrates. To our knowledge, this is the first report of a subtle alternative splicing event giving rise to a significantly different protein isoform, suggesting the existence of complex regulatory mechanisms regarding the expression of the human RNase κ gene.

O 7

Unraveling the signaling pathways of endothelial shear-stress sensing using zebrafish.

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The heart is one of the first organs to form and function during development. Cardiac valves derive from a subset of endocardial cells to prevent retrograde blood flow. These cells undergo elaborate morphogenesis while the heart is functioning and blood flows through the endocardium. The zebrafish heart provides a powerful *in vivo* system to study the interactions of heart function, intracardiac flow dynamics and valve morphogenesis.

We are interested to identify the molecular pathways involved in the shear-stress sensing of endocardial cells during cardiac valve development. To this effect, we interfered with cardiac function pharmacologically and used mutants with impaired heart development. Using RNASeq analyses, we were able to shed light on the shear-stress-mediated transcriptional response induced in whole embryos and from isolated embryonic endothelial cells. These results will help us to understand how the endothelial cells sense and respond to shear stress.

A number of signaling pathways identified in our experiments and their putative involvement in valve development will be discussed during our presentation. Our data will help elucidate how gene networks cooperate to shape heart valves.

O 8

Nuclear receptor NR5A2 regulates proliferation and differentiation of neural stem cells during development through distinct mechanisms

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Nuclear receptors (NRs) play key roles in central nervous system (CNS) development and function. Among them, NR5A2 (LRH-1), an orphan NR, has been recently reported to be highly expressed in CNS. Despite this finding and its involvement in stem cell pluripotency, embryogenesis, tumorigenesis, metabolism homeostasis, steroidogenesis, development and function of many other tissues and organs, the physiological role of NR5A2 in CNS still remains largely elusive. We have only recently shown that NR5A2 is involved in the regulation of Notch signaling during neuronal differentiation (Kaltezioti et al, 2010, *PLoS Biol*; Stergiopoulos & Politis, 2013, *Arch Biochem Biophys*). Here, we provide functional evidence that NR5A2 is a critical regulator of CNS development by controlling proliferation and differentiation decisions in neural stem cells (NSCs). By expression studies, we showed that NR5A2 is specifically associated with the neuronal lineage in CNS of vertebrates. In agreement, gain- and loss-of-function experiments in primary NSCs and analysis of knock-out mice embryos suggest that NR5A2 strongly arrests proliferation, induces neurogenesis and blocks astrogliogenesis. Mechanistically, NR5A2 induces neuronal differentiation via a direct action on the promoter of *Prox1* gene, and blocks proliferation through a direct binding and transcriptional de-repression of *Cdkn2a* (p16) and *Cdkn2b* (p15) genes of the *INK4/ARF* locus. Collectively, these observations, together with the recently discovered pharmacological agonists/antagonists of NR5A2, render it a candidate target gene for regenerative medicine and treatment of nervous system-related diseases and cancers.

O 9

MLP binding to cMyBP-C is important for myoblasts differentiation to myotubes

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Muscle Lim Protein (MLP) is a multifunctional scaffold protein implicated in both cardiac and skeletal muscle physiology and pathophysiology. The full spectrum of its interactions and molecular roles is under-explored.

Using the yeast two-hybrid system (Y2H) we determined that the cardiac myosin-binding protein C (MyBP-C), a structural and regulatory component of the sarcomeric myosin thick filament, is a novel binding partner of MLP. The minimal interacting domains were identified as the inter-LIM domain of MLP and the C4 immunoglobulin domain of MyBP-C by Y2H, *in vitro* binding and blot overlay assays using MBP and GST fusion constructs, respectively. The interaction was confirmed with pull down assays using cardiac and skeletal muscle homogenates and was found to be reduced by MyBP-C phosphorylation and low Ca^{2+} concentrations (pCa>6). Immunofluorescence studies of different C2C12 mouse myoblasts differentiation stages as well as of human skeletal muscle revealed that the MLP/MyBP-C interaction occurs at intermediate stages of myoblast to myotube formation before the complete formation of sarcomeres and striations. Transfected C2C12 with the minimal interacting C4 immunoglobulin domain of MyBP-C, a specific inhibitor of endogenous MLP/MyBP-C interaction, exhibit substantial differentiation delay by 65%.

In conclusion, MLP and MyBP-C interact in a spatio-temporally manner and their complex promotes the differentiation of myoblasts to myotubes. The direct association of MLP and MyBP-C, and its impairment in the cardiomyopathy setting, could potentially explain some of the mechanisms of disease pathogenesis.

O 10

GCN-2 signaling and TOR pathway go hand to hand to promote longevity and stress resistance in Caenorhabditis elegans

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Animals respond to multiple environmental stresses by behavioral, metabolic and physiological changes in a coordinated manner. The general control non-derepressible 2 (GCN2) signaling is a nutrient-sensing pathway that responds to amino acids deficiency and induces a genetic program to effectively maintain cellular homeostasis. It was first described in *Saccharomyces serevisiae* but it was found later to be present in mammals, where it can also regulate lipid metabolism, feeding behavior, UV-irradiation response, synaptic plasticity and memory. Activation of GCN2 by uncharged tRNAs results in phosphorylation of eIF2α, inhibition of global protein synthesis and favored translation of specific mRNAs, such as that encoding the transcription factors GCN4 in yeast or ATF4 in mammals. We established the conserved role of *C. elegans* GCN-2 under amino acid limitation as a translation initiation factor 2α (eIF2α) kinase. Using a combination of genetic and molecular approaches we showed that GCN-2 plays a central role in survival under nutrient stress and mediates lifespan extension conferred by dietary restriction (DR) or inhibition of the major nutrient-sensing pathway, the target of rapamycin (TOR). We also demonstrated that the GCN-2 and TOR signaling pathways converge on the PHA-4/FoxA transcription factor and its downstream target genes to ensure survival of the whole organism under a multitude of stress conditions, such as nutrient scarcity or environmental insults. This is one step forward in the understanding of evolutionary conserved mechanisms that confer longevity and healthspan

O 11

Enhanced proteasome degradation extends Caenorhabditis elegans lifespan and decelerates aggregation-related pathologies

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Aging and various age-related neurodegenerative disorders are associated with decline in proteostasis and accumulation of damaged macromolecules. The proteasome is the major cellular protease implicated in the disposal of normal and damaged proteins, having an impaired function during aging. In previous reports using human primary cells, we demonstrated that proteasome activation through overexpression of proteasome subunits confers extension of replicative senescence and resistance to oxidative stress. In this study, we employed a multicellular model organism, *Caenorhabditis elegans*, to investigate the impact of enhanced proteasome function on lifespan and aggregation-related pathologies. We have found that overexpression of a single core proteasome subunit in wild type worms resulted in enhanced proteasomal function and increased animal longevity and survival under proteotoxic conditions. The lifespan extending effect of the ectopic expression of the core proteasome subunit was found to depend on the FOXO transcription factor DAF-16 and was associated with its elevated transcriptional activity. Finally, we have unveiled a major impact of enhanced proteasome activity on pathologies associated with the accumulation of protein aggregates in surrogate nematode models of neurodegeneration. Understanding the mechanism by which preservation of proteostasis, via increased proteasome function, decelerates the aging process and protects against age-related pathologies may lead to new therapeutic and anti-aging interventions.

O 12

Functional involvement of the Nrf2/Keap1 signaling pathway in the age- and tissue-dependent regulation of proteasome in Drosophila

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Proteasome is central to cellular proteostasis (homeostasis of the proteome) maintenance as it degrades both normal and damaged proteins. Following a detailed analysis of proteasome regulation in the *in vivo* setting of *Drosophila melanogaster* we found that a major hallmark of flies' somatic tissues aging is the gradual accumulation of ubiquitinated and carbonylated proteins; these effects correlated with a ~50% reduction of proteasome expression and catalytic activities. In contrast, aging flies' gonads were relatively free of proteome oxidative damage, and maintained substantial proteasome expression levels and highly active proteasomes; also, gonads of young flies were found to possess more abundant and more active proteasomes than somatic tissues. Furthermore, the gonads were, independently of age, more resistant than soma to oxidative challenge and, as analyses in reporter transgenic flies showed, retained functional antioxidant responses. Finally, inducible activation of endogenous Nrf2 (a master regulator of cellular antioxidant responses) in transgenic flies promoted youthful proteasome expression levels in the aged soma, while RNAi-mediated Nrf2 knockdown resulted in aged gonads with reduced proteasome functionality. These observations indicate that age-dependent Nrf2 dysfunction is causative to decreasing somatic proteasome expression during aging. They also support the notion that, given the finite resources that are available during the lifetime, the distinction between somatic and reproductive tissues necessitates that the "immortal" germ line is maintained at a functional level that preserves viability across generations, whereas investment in the "mortal" soma is more limited, as it is destined merely to support the survival of a single generation.

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O 13

A holistic evolutionary and structural study of Flaviviridae provides insights into the function and inhibition of HCV helicase

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Viruses of the *Flaviviridae* family are the causative agents of many common and devastating diseases, including hepatitis, yellow fever and dengue fever. As there is currently no available anti-*Flaviviridae* therapy, there is urgent need for the development of efficient antiviral pharmaceutical strategies. Viral RNA helicases are involved in duplex unwinding during the RNA replication of the virus. These helicases represent very promising antiviral targets. Herein, we report a complete phylogenetic analysis of RNA helicases across *Flaviviridae*, alongside an in-depth evolutionary analysis which revealed a series of conserved and invariant amino acids that are predicted to be key to the function of the helicase. Structural molecular modelling revealed the strategic significance of these residues based on their relative positioning on the 3D structures of the helicase enzymes, which may be used as pharmacological targets. We previously reported a novel series of highly potent HCV helicase inhibitors, and we now reassess their antiviral potential using the 3D structural model of the invariant helicase residues. We find that the most active compound of the series, compound C4, exhibited an IC50 in the submicromolar range, whereas its stereoisomer (compound C12) was completely inactive. Useful insights were obtained from molecular modelling and conformational search studies via molecular dynamics simulations. C12 tends to bend and lock in an almost "U" shape conformation, failing to establish vital interactions with the active site of HCV. On the contrary, C4 spends most of its conformational time in a straight, more rigid formation that allows it to successfully block the passage of the oligonucleotide in the ssRNA channel of the HCV helicase. This study paves the way and provides the necessary framework for the in-depth analysis required to enable the future design of new and potent antiviral agents.

O 14

Effect of non-contact current transfer in protein crystallization: experiments with two model proteins

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The growth of protein crystals, suitable for X-ray crystallography is known to be the basic element of the protein structure determination. Crystallographers face problems such as the amount of the protein, the availability of high-quality protein crystals and the control of the crystal size in a daily basis. Many efforts have been made to improve crystallization process with the use of magnetic fields, electro-focusing, and internal or external electric fields. Electric fields are a significant methodological advancement and have been used in order to enhance nucleation and crystal growth. In this work, the influence of a non-contact current transfer upon the crystallization of two model proteins -lysozyme and RNase A-, was investigated using a prototype ion-generator device. The device is capable of producing an equivalent of microcurrent that can be transmitted wirelessly by spraying negatively charged ions using available air gases (O_2) as a transfer medium. For our crystallization experiments the OryxNano robot was used in a sitting drop vapour diffusion setup. From a crystallographic point of view, non-contact current transfer clearly enhances crystal formation process of the two proteins, in terms of speed and quality of crystal formation. The crystal quality and the three-dimensional structure of lysozyme grown with and without the electric field influence was analysed by means of X-ray diffraction. Our results indicate a considerable positive effect on the quality of the crystal diffraction pattern after non-contact current transfer, pin pointing the great value of the technique and its potential implementation in routine protein crystallization.

O 15

Modeling, substrate docking and mutational analysis identify residues essential for function and specificity of a major fungal purine transporter

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The AzgA purine/H^+ symporter of the filamentous ascomycete *Aspergillus nidulans* is the founding member of a functionally and evolutionary distinct transporter family present ubiquitously in fungi, several bacteria and plants. Here we show that a valid AzgA topological model can be built based on the crystal structure of the *Escherichia coli* uracil transporter UraA, which is member of the Nucleobase-Ascorbate Transporter (NAT/NCS1) family. The model consists of 14 transmembrane α-helical, segments (TMSs) and cytoplasmic N- and C-tails. A distinct compact core of 8 TMSs, made of two intertwined inverted repeats (TMS1-4 and TMS8-11), is topologically distinct from a flexible gate domain (TMS5-7 and TMS11-14). A putative substrate binding cavity is visible between the core and the gate domains. Substrate docking approaches and mutational analysis led to the identification of residues essential for substrate binding and/or transport (Thr49, G129, N131, Asp342, Glu394 and Ser395). These results support the idea that AzgA is a distant member of the NAT family. Given the lack of mammalian AzgA homologues and the presence of essential AzgA-like transporters in microbial pathogens, we discuss the possibility of using AzgA homologues as specific targets or gateways of highly targeted antimicrobials.

O 16

Structural and functional studies on La and RRM1 motifs of an important tRNA-associated chaperone

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La/SSB protein is an important chaperone of RNA polymerase III transcripts, including premiRNAs. It guides accurate maturation of pre-tRNAs, while protecting them at the same time from random degradation. La/SSB is found in abundance within nuclei, and studies have shown that it is up-regulated during oncogenesis, a cellular perturbation which requires elevated protein synthesis rates. Originally discovered in patients with systemic lupus erythematosus and Sjogren's syndrome, it contains a characteristic conserved, predominantly helical structure, termed "La motif" and two RNA binding motifs (RRM1 and RRM2). So far, the structure of full-length La protein remains elusive. Scattered structural data of the La and the RRM motifs from few eukaryotes (including human) provide only limited information on the possible alternative roles of La/SSB protein in a more dynamic tRNA-dependent cellular network. Therefore we initiated an extensive structural and functional characterization of a "domain library" of La/SSB which includes several domains of different length and content. Here, we present the NMR-derived structures of La and RRM1 motifs. Both motifs were found to be well-folded in "winged-helix" and classical RRM structures, respectively, as revealed by high resolution NMR spectroscopy. In addition, the RNA binding properties of the La motif were investigated and the interaction interface was identified through chemical shift perturbation of amide groups in 1H-15N HSQC spectra. Surprisingly, both biochemical and NMR analysis revealed that La motif can mediate specific protein-tRNA interactions, which has never been reported before and raises questions on putative hidden specificities of La/SSB protein.

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O 17

Sugar vs sugar: the crystal structures of the first efficient xylose derivatives for the inhibition of glycogen phosphorylase determined at high resolution

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Pursuing new therapeutic interventions for type 2 diabetes has directed research efforts towards a more thorough investigation of the existing information to ensure that maximum knowledge is derived. This odyssey has been aided by X-ray crystallography, an irreplaceable tool to elucidate the structure function relationships underlying macromolecular targets involved in the disease. In this context, the distinct binding sites of glycogen phosphorylase (GP), responsible for glucose homeostasis through glycogen metabolism have been probed with a number of compounds. The most intensively investigated of those have been derivatives of glucopyranose (GPIs) that were shown to achieve strong inhibition of the enzyme activity when large aryl groups were introduced in the aglycon. This was attributed to the favourable contacts formed in the so-called β-pocket of the active site with the residues lining this subsite as revealed by their 3D structure. GPIs could hence, be considered as potential means for therapeutic intervention in type 2 diabetes as well as some other diseased states (e.g. early cardiac and cardiovascular disease in non-diabetics, cardiac arrhythmias, ischemic injuries, tumour growth) [1-2]. With the aim to further investigate the effect of the sugar configuration a series of xylose derivatives were prepared with aglycons. The crystal structure of two of those compounds were determined at high-resolution (1.63 Å and 1.7 Å) using synchrotron radiation at beamline P14, Petra III, EMBL-Hamburg Unit giving for the first time new insights on the importance of the sugar moiety in the binding affinity of the ligands and the structure-based design of new antidiabetics.

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O 18

Aldh1b1 regulates the timing in pancreas lineage segregation and beta cell maturation

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Aldehyde dehydrogenase activity is increasingly associated with stem and progenitor cells but its functional significance remains uncertain. We identified Aldh1b1 as a marker for pancreas progenitor cells. Aldh1b1 is exclusively expressed in the emerging pancreatic buds in a Pdx1 dependent manner. Subsequently, Aldh1b1 is expressed in both the tips and trunks of the developing pancreatic epithelium and its expression in the latter is Ngn3 dependent. In the adult, Aldh1b1 persists in very rare centroacinar-like cells but the number of Aldh1b1+ cells expands greatly during the regenerative response that follows streptozotocin or caerulein treatment. Thus Aldh1b1 expression is associated with pancreas stem and progenitor cells during development and in the adult. To address the role of Aldh1b1 in pancreas development we generated a lacz knock-in mouse strain. Aldh1b1 null mice are viable and fertile but defects in pancreas morphogenesis are evident already during embryo development. The timing of the emergence of endocrine progenitors and differentiated acinar and ductal cells is altered in the null embryos. In newborn null animals, islet morphology and insulin expression are disrupted and this is not corrected during postnatal development. Adult null mice display a disruption of islet architecture, lower ratio of mature to immature secretory granules in the beta cells as well as associated glucose intolerance and age dependent onset of hyperglycaemia. Therefore, Aldh1b1 regulates the timing of pancreas lineage specification and is important for subsequent functional maturation of beta cells. Incomplete maturation manifests later in life with hyperglycaemia and glucose intolerance

O 19

miR-21 suppresses SOX2 and regulates human mesenchymal stem cell properties

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MicroRNAs (miRNAs) have recently been shown to act as regulatory signals for maintaining stemness and determining the fate of adult and fetal stem cells, such as human mesenchymal stem cells (hMSCs). hMSCs constitute a population of multipotent stem cells that can be easily expanded in culture and are able to differentiate into many lineages. We have isolated two subpopulations of fetal MSCs from amniotic fluid (AF) known as spindle (SS) and round-shaped (RS) cells and characterized them based on their phenotypes, pluripotency, proliferation rates and differentiation potentials. In this study, we analyzed the miRNA profile of MSCs derived from AF, bone marrow (BM) and umbilical cord blood (UCB). We initially identified 67 different miRNAs that were expressed in all three types of MSCs but at different levels, depending on the source. A more detailed analysis revealed that miR-21 was expressed at higher levels in RS-AF-MSCs and BM-MSCs compared with SS-AF-MSCs. We further demonstrated for the first time a direct interaction between miR-21 and the pluripotency marker Sox2. The induction of miR-21 strongly inhibited Sox2 expression in SS-AF-MSCs, resulting in reduced clonogenic and proliferative potential and cell cycle arrest. Strikingly, the opposite effect was observed upon miR-21 inhibition in RS-AF-MSCs and BM-MSCs, which led to an enhanced proliferation rate. Finally, miR-21 induction accelerated osteogenesis and impaired adipogenesis and chondrogenesis in SS-AF-MSCs. Therefore, these findings suggest that miR-21 might specifically function by regulating Sox2 expression in human MSCs and might also act as a key molecule determining MSC proliferation and differentiation.

O 20

HP1 dynamics reports a continuum of chromatin states in pluripotent and terminally differentiated cells.

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Differences in the diffusional mobility of heterochromatin protein-1 (HP1) and other chromatin-associated proteins are often interpreted in the context of the euchromatin-heterochromatin divide, which assumes that loosely and tightly packed chromatin contain distinct anchorage sites and represent two fundamentally different microenvironments. However, accumulating information now suggests that the diffusion rates of HP1 within similarly compacted domains vary more than three orders of magnitude, depending on cell type and differentiation state. Prompted by these findings, we have revisited HP1 dynamics, monitoring the behaviour of two HP1 variants (HP1α and HP1γ) in mouse embryonic stem cells and wild type or ichthyosic fibroblasts. Results obtained by fluorescence recovery/loss after photobleaching (FRAP/FLIP) show that HP1 dynamics exhibit significant variation at both the ensemble (cell population) and the single-cell/single-aggregate level. Correlation of FRAP/FLIP data with results obtained by genome-wide microarray screens, quantitative PCR and quantitative immunofluorescence strongly suggest that, beyond the differences between euchromatin and heterochromatin, the variable dynamics of HP1 reflect a rolling continuum of chromatin states. Interestingly, this "dynamic plasticity" is more obvious in heterochromatic regions than in euchromatic areas of the cell nucleus, indicating that developmental transitions concern primarily the remodelling of compacted chromatin.

O 21

Two neurogenic factors, Cend1 and Neurogenin-2, drive astrocytic and MEFs' reprogramming towards multipotency and neurogenesis

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Recent studies demonstrate that astroglia isolated from non-neurogenic brain regions has the potential to be reprogrammed into functional neurons through forced expression of factors instructing neurogenesis. Based on our previous studies on the potential of the neurogenic gene Cend1 in directing NSCs to exit the cell-cycle and acquire a neuronal phenotype, in parallel with evidence demonstrating Cend1 activation by genes of the neurogenin family, we explored the reprogramming potential of Cend1 and Neurogenin-2 on postnatal cortical astrocytes. To this end, forced expression of either Cend1, Neurogenin-2 or both, resulted in trans-differentiation of astrocytes towards the neuronal lineage, as they were exhibiting differentiated neuronal morphology and expressed β-III-tubulin and neuronal subtype-specific markers, GABA and TH.Only in double-transduced cultures, Cend1$^+$/Ngn2$^+$ astrocytes formed high proliferating spheres (astrospheres) of Glast$^+$/Nestin$^+$ cells, which, in the absence of growth factors, differentiated into neurons, astrocytes and oligodendrocytes, implying their neural stem cell-like properties. In order to investigate whether Cend1 and Ngn2 have a broader neurogenic potential and are capable of trans-differentiating more distant in lineage cell types, we transduced mouse embryonic fibroblasts (MEFs) with lentiviral vectors expressing the two molecules. Our results indicate that forced expression of Cend1, Ngn2 or both resulted in MEFs reprogramming, initially towards neural progenitors and at later stages towards neurons. This finding demonstrates that common reprogramming mechanisms exist instructing neuronal trans-differentiation of different cell types. It also highlights the existence of a group of common factors that inactivates the differentiated cell program and activates genes associated with NSCs proliferation and differentiation state.

O 22

Cloning, expression, purification and biological activity of Human Soluble Stem Cell Factor (hr sSCF) in E.Coli cells

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Human Stem Cell Factor (SCF) is a proinflammatory cytokine and a growth factor for various biological systems, including the hematopoietic system. SCF forms dimmers and activates its transmembrane RTK (Receptor Tyrosine Kinase) receptor, c-Kit. SCF can be found either in a transmembrane (mSCF) or in a soluble form (sSCF). Recombinant human soluble SCF is important not only as a research tool but also as a biotherapeutic due to its usage, along with G-CSF, in the *ex vivo* expansion of hematopoietic stem cells. Here, we present the cloning and the production of human recombinant sSCF as well as an assessment of its biological activity. The CDS region of the human SCF was isolated from total mRNA of placental origin and was cloned into the fusion plasmid vector pCR2.1. The CDS region of the human sSCF was isolated from the recombinant plasmid pCR2.1-SCF and was cloned in the fusion plasmid expression vector pET-16b. C43 (DE3) E.*Coli*, transformed with the recombinant plasmid pET-16b-sSCF, expressed the recombinant sSCF. The recombinant sSCF protein was solubilized from the inclusion bodies using L-Arginine 1M. It is believed that L-Arginine also properly refolded the protein of interest. Recombinant sSCF was added in c-Kit$^+$ K562 cell suspension culture and found to be biologically active, by augmenting cell growth, diminishing cell death and increasing the rate of DNA synthesis

O 23

Correlation between growth, survival and gene expression boundaries of Listeria monocytogenes in response to acid and osmotic stress

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Listeria monocytogenes is known to possess multiple adaptive response mechanisms that assist the organism in surviving under extremely stringent conditions. Little, though, is known about the physiology and particularly the transcriptional changes that occur in *L. monocytogenes* under conditions marginal for growth. Hereby, we aimed to correlate the expression levels of stress- (*gad2, sigB*) and virulence-associated genes (*prfA*), following habituation under suboptimal acid and osmotic conditions with subsequent resistance to lethal acid stress (pH 2.0 with HCl). Two strains of *L. monocytogenes* (serotypes 4b, 1/2a) were independently inoculated in culture media containing various combinations of NaCl (0-10% w/V) and pH (4.8-6.4) and stored at 7oC for up to 10 days.

Results showed that *L.monocytogenes* strains could differ in their growth and/or survival limits, regarding pH, NaCl presence and storage temperature, and the resulted interface is significantly affected by storage time. Furthermore, monitoring the changes in gene expression during storage, pointed out a possible association of boundaries in expression of virulence and stress related genes with habituation under suboptimal acid and osmotic stress. High survival, as manifested by low log reductions and high DpH=2.0 values after exposure to pH 2.0, coincided with *sigB* upregulation and high *gad2* expression levels after 2 or 6 days of habituation under the aforementioned sub-optimal pH and NaCl levels. Conversely, low survival was correlated with sigB down-regulation and lower *gad2* expression compared to the other cases. These trends in gene expression suggest that either the direction or the level of *sigB* and *gad2* expression may correlate with the growth boundaries and acid resistance of *L. monocytogenes*. Notably, habituation to all conditions and subsequent exposure to extreme acid stress always induced virulence (*prfA*) gene expression, suggesting the stimulatory effect of acid and osmotic stress to virulence of this pathogen.

Overall, mapping the molecular physiology of pathogen in probability terms (i.e., genes switch on vs switch off) as described by gene expression/no expression interface, along with phenotypic observations could facilitate the accurate stochastic assessment of the virulence potential of the organism, under conditions that may be encountered during food manufacturing and storage.

O 24

Engineering bacteria for the discovery of potential therapeutic compounds against protein misfolding diseases

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It has now been widely recognized that a variety of serious human diseases with an enormous socioeconomic impact, such as Alzheimer's disease, Parkinson's disease, certain types of cancer, type II diabetes, etc., are initiated by a common mechanism: the misfolding of specific proteins. Today, no therapeutic treatments exist for the vast majority of these disorders. Here, we describe the use of engineered bacterial cells as a platform for the discovery of potential therapeutics against such protein misfolding diseases (PMDs). The topic of the described research is the directed evolution of small molecules with the ability to bind to and restore the problematic folding of PMD-associated proteins. To achieve this, *Escherichia coli* cells are first engineered to biosynthesize large libraries of test compounds exhibiting high levels of chemical and structural diversity. Then, the same cells are modified further so that they allow the identification of the rare molecules with the ability to bind to and correct the folding of particular misfolding-prone and PMD-associated proteins (MisPs) with the use of a genetic screen. Lead compounds identified by this initial screen, are then subjected to more detailed evaluation by biochemical and biophysical methods of protein analysis, and their ability to inhibit MisP-induced pathogenicity is tested using appropriate human cell assays or *in vivo* models of the disease of interest. The molecules capable of rescuing the misfolding of the target MisP and of antagonizing its associated pathogenicity become drug candidates against the specific disease. We will describe our efforts to identify such "pharmacological chaperones" against the misfolding of the amyloid β (Aβ) peptide and of certain carcinogenic misfolded variants of human p53, with the aim of developing potentially therapeutic compounds against Alzheimer's disease and cancer, respectively

O 25

Food related streptococci beyond Streptococcus thermophilus: friends or foes? A comparative genomics approach

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Streptococcus thermophilus is the only *Streptococcus* used in food fermentations which during its adaptation to the milk environment evolved through loss-of-function events resulting in a diminished pathogenic potential. Nevertheless, additional streptococcal species like *Streptococcus macedonicus* and *Streptococcus infantarius* can be found in fermented foods especially of dairy origin. Both species belong to the *Streptococcus bovis/Streptococcus equinus* complex (SBSEC), formerly known as *S. bovis* group. Members of the SBSEC have been associated with human disease including endocarditis and colon cancer. Here we perform comparative genomics among dairy and clinical SBSEC members with complete genome sequences. *Streptococcus macedonicus*, *S. infantarius* and *Streptococcus pasteurianus* seem to have undergone reductive evolution resulting in significantly diminished genome sizes and increased percentages of potential pseudogenes when compared to *Streptococcus gallolyticus* subsp. *gallolyticus*. In addition, the three species seem to have lost genes for catabolizing complex plant carbohydrates and for detoxifying toxic substances previously linked to the ability of *S. gallolyticus* to survive in the rumen. Both *S. macedonicus* and *S. infantarius* have features that could support adaptation to milk, including extra gene clusters for lactose and galactose metabolism and loci acquired through horizontal gene transfer with potential donors the dairy *Lactococcus lactis* and *S. thermophilus*. In addition, the two species lack several pathogenicity-related genes found in *S. gallolyticus*, like the two pilus operons *pil1* and *pil2*. Clearly, *S. macedonicus* and *S. infantarius* present traits of adaptation to milk and a diminished pathogenic potential, however more research is needed before they can be used in food fermentations.

O 26

Identification and cloning of all two-component systems from Thermus thermophilus HB8 and their molecular cross-talk

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Prokaryotes employ relatively simple signal transduction systems, which usually consist of one or two proteins, to adapt to internal and external stimuli. Two-component systems (TCSs) typically consist of a transmembrane sensor histidine kinase (HK) receptor and a response regulator (RR), which is activated via histidine-aspartate phosphorelay. Since the complete genome of *Thermus thermophilus* HB8 has been sequenced, 11 HKs and 14 RRs were identified based on the comparative study of several databases involving genome and protein family domain annotations. The genes of all TCSs are located on the bacterial chromosome except for a cognate pair that is found on the pTT27 megaplasmid. For the functional characterization of all TCSs *in vitro*, the full-length coding sequences of the 14 RRs and 11 HKs were amplified. Eight of the HKs were constructed at their truncated form by removing the predicted amino-terminal transmembrane regions. Each amplified sequence was cloned into pET-29c vector and purification of the overexpressed proteins was carried out by affinity metal chromatography. Results from *in vitro* phosphorylation assays revealed the communication of the cognate pairs, as well as other less intense interactions, raising the possibility that the cross-talk in signal transduction takes place between TCSs in *T. thermophilus*.

O 27

Genetic and antigenic characterization of influenza viruses circulating in Southern Greece during the winter period of 2012-2013

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Influenza viruses are characterized by a unique genome structure, causing genetic instability, especially to the genes of haemagglutinin (HA) and neuraminidase (NA). This study aimed to identify the antigenic and genetic variation of influenza viruses circulated in Southern Greece during the 2012-2013 winter period. Respiratory specimens from influenza-like illness cases were sent weakly to the National Influenza Reference Laboratory of Southern Greece for influenza identification. Representative isolates were further compared to the vaccine components for antigenic relatedness by haemagglutination-inhibition (HI) method. For genetic characterization several overlapping set of primers spaning the whole length of HA and NA genes were used. Phylogenetic analysis was constructed using the neighbour-joining approach implemented in MEGA 5.0. Influenza presence was confirmed in 337 of 1173 cases (28.7%) and included 201 A(H3N2), 121 A(H1N1)pdm09 and 15 influenza B viruses. Phylogenetic analysis of 48 influenza A strains indicated clustering within group 3C of the Victoria/208 clade for the A(H3N2) and within groups 6 and 7 for the A(H1N1)pdm09 viruses, respectively. B viruses clustered within group 2 of the Yamagata lineage. Sequencing of the influenza A strains revealed unique amino acid variations in all antigenic sites of the HA glycoprotein. Variations were also observed in the N-linked glycosylation sites. Antigenic characterization indicated that all isolates showed sufficient reactivity with antisera raised against the vaccine viruses. Our findings confirm the genetic instability of influenza viruses and highlight the importance of continuous molecular surveillance as the basis of effective management of influenza epidemics, as well as for vaccine evaluation.

O 28

Investigating the role of the Histidine Decarboxylase Gene in Tourette Syndrome etiology

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Gilles de la Tourette Syndrome (TS) is a childhood onset neurodevelopmental disorder with complex genetic background that manifests as multiple motor and vocal tics and high comorbidity rates with other neurodevelopmental disorders. While previous studies suggest that genetic and environmental factors contribute to the etiology of the disorder, its pathophysiology remains largely unknown. Recent studies report the possible implication of the histamine decarboxylase (HDC) gene, which is the key enzyme in histamine production, suggesting histaminergic dysfunction may play a role in the etiology of TS. We recently investigated variation across the *HDC* gene for association with TS by interrogating 12 tagging SNPs (tSNP) across the region in a large sample of 520 nuclear families originating from seven European populations as well as Canada. Strong over-transmission of alleles at two SNPs (rs854150 and rs1894236) in the joint dataset was observed, as well as statistically significant associated haplotypes. Using a subset of these samples, we sequenced the complete HDC gene in 383 individuals with TS using next generation high-throughput sequencing technology. Upon analysis of the results using VAAST software we identified novel and known variants in the coding region of HDC gene. The majority of the TS patients carrying these variants had been previously found to carry at least one HDC tSNP significantly associated with TS. Further investigation of the frequency of the identified coding variants in ethnically-matched control individuals is required in order to elucidate the role and relative contribution of the histaminergic pathway in TS etiology

O 29

Mitochondrial tRNA mutations association with Alzheimer's disease

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A number of studies suggest that mitochondrial dysfunction plays an important role in the pathogenesis of Alzheimer disease. In mitochondria of AD patients have been observed several changes both in their structure and function which lead to overproduction of ROS and reduced energy production resulting in neuron damage. To shed light on the role of the mitochondrial genome in the etiology of Alzheimer we analyzed the mitochondrial tRNA genes and part of their flanking regions in 100 patients with AD compared with a set of 204 healthy controls. Even though, tRNA genes consist of 10% of mitochondrial genome, they are characterized by high mutation frequency resulting in synthesis of proteins creating pathogenic phenotypes. We found a total of 48 mutations in 71 out of 100 AD patients. 27 of 48 mutations were not found in control group, 21 of 48 were observed at a significantly lower frequency (p=0,0172), while none of the combinations of mutations detected in AD patients were observed in the controls. Some of these mutations classify patients in different haplogroups, U (6%) and K (4%), in which none of controls are being classified. 9 out of the 48 mutations have already been related to other mitochondrial diseases, while 8 have not been identified before. Our study shows that mitochondrial tRNA mutations, along with specific rRNA, ND1 and ND3 mutations, are significantly more frequent in AD patients than in the controls and is the accumulation of a subgroup of mutations, so the mtDNA genetic backgrounds that are correlated with AD rather than individual mutations.

O 30

The role of insulin and insulin resistance in macrophage activation and M1/M2 polarization

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Obesity is characterized by low-grade inflammation and increased risk of infection, but not with worse outcome of patients with in sepsis and acute lung injury. Hyperinsulineamia and insulin resistance, are commonly found in obese patients. Macrophages, key components of innate immunity are associated with obesity-related pathologies, and metabolic factors, like insulin, directly act on their function. In this study, we evaluated the role of insulin and resistance on macrophage activation and polarization. Exposure of thioglycolate-elicited macrophages from wild type (WT) mice to high concentration of insulin rendered them insulin resistant. Insulin resistant macrophages had reduced responses to LPS in terms of pro-inflammatory cytokine production and expressed elevated levels Arginase-1, Fizz-1 and Ym-1, markers of M2 polarization. Similarly, macrophages from Akt2-/- mice, from mice lacking IGF1R from macrophages ($LysMCre^{+}/IGF1^{fl/fl}$), or from mice fed with High Fat Diet (HFD), were also insulin resistant and obtained M2 characteristics, being upregulation of Arg1, Ym1 and Fizz1. In the polymicrobial sepsis model of cecal ligation and puncture (CLP) mice fed with HFD had reduced levels of pro-inflammatory cytokines in the BALF and alveolar macrophages expressed elevated levels of M2 markers, compared to mice fed with ND. These data suggest that insulin resistance promotes an anti-inflammatory M2 macrophage phenotype and reduces systemic and pulmonary inflammation in response to polymicrobial sepsis.

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O 31

The miR-224-KLK15 axis in prostate tumors: Deregulation and prognostic significance

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MicroRNAs are largely implicated in the pathogenesis of prostate cancer by targeting key tumor-related genes, such as kallikreins (*KLKs)*. The present study aims to concurrently analyze the expression of miR-224 and its target, *KLK15*, in prostate tumors in order to reveal any translationally relevant deregulations. Total RNA was extracted from prostate cancer (CaP) cell lines and prostate tumor tissues. MiR-224 and *KLK15* expression levels were measured using optimized qRT-PCR methods. Interestingly, *KLK15* was heavily overexpressed in malignant and more advanced prostate tumors, whereas miR-224 was significantly downregulated in the aforementioned biological settings. A weak inverse correlation was observed in benign tumors, which was significantly strengthened in CaP samples (r_s=-0.434, p<0.001), indicative of an *in vivo* deregulated interaction. A promising prognostic potential was identified both for *KLK15* (HR = 3.36, p = 0.038) and miR-224 (HR=0.314, p = 0.013). The miR-224-*KLK15* axis can prove to provide helpful translational applications for CaP and, according to our work in progress, for other major urological malignancies.

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O 32

Establishment of a 3-Dimensional Differentiated Hepatocyte Culture System for the Investigation of the Role of MicroRNAs in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) has been widely studied in 2D models using cultured cancer cells growing as a monolayer. 3D *in vitro* models have been used as a compromise between 2D cultures of isolated cancer cells and the manufactured complexity of human cancer xenografts. HNF4α transcriptional silencing contributes to HCC formation through a differentially altered microRNA (miRNA) profile. This study aims to create a more biomimetic 3D HCC model and find correlations between cell phenotypic behavior and gene expression changes of specific miRNAs (mir24) that contribute to HCC carcinogenesis and metastasis. Huh7 cells cultured in PolyHema-coated surfaces form spheroids up to 100 μm in diameter within 72hr and up to 1 mm with long-term culture (10 days), with distinct architectural features reminiscent of the *in vivo* state. HCC spheroids were characterized by immunohistochemistry for the expression of tissue specific markers such as E-cadherin, HEPAR1, α-fetoprotein, glypican -3 and glutamine synthetase. Viability and apoptosis were assessed by Ki67 and Bcl-2 staining, respectively. Comparative analysis of the malignant phenotype of HCC spheroids with miR24-mediated HNF4α silencing with corresponding antagomir and negative controls, has shown significant increase in proliferation, migration and invasion properties, supported also by Live/Dead viability assays. Real-time quantitative PCR arrays are being used to assess the biological effects of miR24-related HNF4α deregulation on epithelial-to-mesenchymal transition related networks of Notch and TGF-β signaling. This well-defined *in vitro* microenvironment provides a versatile tool to investigate hepatic cell-extracellular matrix and cell-cell interactions, in relation to the biological effects of HNF4α – related miRNAs deregulations.

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O 33

How might alterations of histone acetyltransferases contribute to Urothelial Cell Carcinoma?

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Bladder cancer is the ninth most common malignancy worldwide. **U**rothelial **C**ell **C**arcinoma (UCC) is the main (90%) histologic type in Western countries. Genetic changes in UCC cover a wide spectrum and some of them are specific for subtypes (papillary vs. muscle-invasive) of UCC. Moreover, there are multiple epigenetic changes. Accumulating evidence indicates that perturbation of histone modifications, prominently acetylation, is associated with cancer development. The causes and effects of these changes are incompletely understood. Recent studies revealed recurrent mutations in the histone acetyltransferases (HATs) CBP/p300 in UCC. Additionally, aberrant expression and deregulated levels of these and other HATs could also lead to malignant transformation. Notably, inactivating mutations often affect only one allele of epigenetic regulator proteins. To date, it is unknown how these alterations affect the properties of UCC. We hypothesize that mutational and expression changes in various HATs create a level of activity optimally conducive to tumor development and progression. Therefore, we have begun to study expression changes in six HATs (MOZ, MORF, CBP, p300, GCN5, PCAF) in UCC using tissue samples from cystectomies and a large panel of UCC cell lines, covering the whole spectrum of the disease, and determine the effects of their experimental up- or down-regulation on essential properties of urothelial cancer cells. This analysis will elucidate the contribution of these HATs to UCC. Moreover, since mutations of CBP/p300 are not restricted to UCC, this analysis will shed light on mechanisms of carcinogenesis in other tumors

O34

An IRES-like element identified within the HCV core-coding region mediates internal translation initiation of the core+1/Short protein in the HCV replicon

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Translation of *hepatitis C* virus (*HCV*) genomic RNA is directed by an *internal ribosome entry site* (*IRES*) in the *5'-untranslated region*. We have previously shown that the two conserved RNA stem-loop structures of the core protein encoding region, SL47 and SL87, are important for HCV genome translation, in cell culture and *in vivo.* Moreover, we have reported that an overlapping the core gene open reading frame (core+1 ORF) encodes alternative translation products including a protein initiated at the internal codons 85/87 (core+1/S). Here, we identified a novel role for SL47 and SL87 as an integral part of an IRES-like element embedded in the 5' end of core region that directs core+1/S internal translation initiation. First, different parts of the 1a and 2a core sequences were tested for their putative IRES activity by insertion in a dual luciferase system and RNA transfection into Huh7 cells combined with site-directed mutagenesis. The results showed the presence of a regulatory element within core nt 344-596 that directs internal translation initiation at core+1/S codons 85/87 and that RNA SL47 and SL87 integrity is essential for this activity. Then, we interestingly demonstrated that in the JFH1 replicon, core+1/S is expressed as the predominant isoform of core+1 protein at early hours of viral translation/replication cycle. Moreover, core+1/S is expressed independently of the viral IRES or polyprotein initiator whereas in high correlation with intact RNA SL47 and SL87. Thus, in the HCV replicon, SL47 and SL87 are necessary elements for the internal IRES-mediated alternative translation of core+1/S protein

O 35

Activation of Corticotropin Releasing Factor receptor type 2 promotes hormone-dependent growth and migration of breast cancer cells

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Cortocotropin releasing factor (CRF) system plays a central role in the regulation of stress responses. In parallel to their pituitary and CNS actions, CRF and its homologues Urocortins I, II and III, exert important direct biological effects in the periphery, via activation of two distinct receptors. CRF1 and CRF2 receptors present different pharmacological profiles to their lignads, and their expression is tissue dependent. Many studies demonstrate that the CRF system could be involved in the growth and progression of human cancer. Recently, we have shown the expression of both CRF receptors in human breast cancer biopsies and evaluated their prognostic/diagnostic potential. In the present study, we investigated the expression of CRF receptors in MCF-7 human breast cancer cell line by immunofluorescence. We then examined the effect of Urocortin II, a specific CRF2 ligand, in combination to the known mitogen 17-β estradiol on MCF-7 growth and migration, using the xCELLigence real-time cell monitoring system. Urocortin II did not alter MCF-7 cell growth by each self, but it enhanced significantly the growth-promoting effect of E2, and this action was reversed by the selective CRF2 antagonist Astressin -2B. On the other hand, Urocortin II caused a dose- and time-dependent increase in the migration potential of MCF-7 cells, which again was inhibited by astressin2B. Urocortin II also enhanced the migration-promoting effect of E2. In conclusion, CRF2 specific activation was shown to increase estrogen-dependent growth and migration potential of breast cancer cells. Further studies will be conducted to unfold molecular events involved in breast carcinogenesis via specific signaling pathways of the CRF system.

O 36

TAK1 plays a central role in NF-kB–dependent IL-8 secretion induced by Helicobacter pylori infection of gastric epithelial cells

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Infiltration of the gastric lamina propria by neutrophil polymorphs is a hallmark of *H. pylori (Hp)* infection in patients and it is partly orchestrated by IL-8 secretion induced by gastric epithelial cells, following infection with strains harboring a functional type IV secretion system (T4SS). Moreover, the bacterial CagA protein which is translocated through the T4SS has been shown to activate NF-kB thus contributing to IL-8 secretion by gastric epithelial cells (AGS). Intracellular CagA is hierarchically tyrosine phosphorylated, by host Src and Abl kinases, on repeating EPIYA motifs namely EPIYA-*A*: GLKN(ST)EPIYAKVNKKK, EPIYA-*B*: Q(V/A)ASPEPIY(A/T)QVAKKVNAKI and EPIYA-*C*: RS(V/A)SPEPIYATIDDLG. Previous work by our group suggested that early NF-kB induction may involve presence of functional EPIYA-C motifs in CagA and did not depend upon Erk1/2 or AKT activation. With the aim to study the role of TAK1 in NF-kB-dependent IL-8 secretion during *Hp* infection, we infected AGS cells with isogenic mutant strains expressing CagA with variable EPIYA-C phosphorylation motifs (n=0-3) and their respective EPIFA phosphorylation deficient counterparts and determined levels of secreted IL-8. In the presence of specific TAK1 inhibitor 5Z-7-Oxozeaenol, a concentration-dependent arrest of IL-8 production was observed during *Hp* infection, irrespective of the CagA status of phosphorylation. Furthermore, when we infected TAK1-/- mouse embryonic fibroblasts (MEFs) and their respective control MEFs with the *Hp* mutants, we observed NF-kB activation, only in the control MEFs, independent of the expression or the phosphorylation status of CagA. These results suggest that TAK1 plays a critical role in the induction of the inflammatory process during *Hp* infection.

O 37

Changes in the transcriptional profile of nucleus pulposus intervertebral disc cells under hyperosmotic conditions

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Nucleus pulposus intervertebral disc cells are routinely confronted with high osmolality. We have shown that hyperosmotic stress reduces cellular proliferation by activating a p38-mediated G2 and a p53-dependent G1 cell cycle arrest, exerting a genotoxic effect. In contrast to several previous reports concerning renal cells, nucleus pulposus cells residing within a hyperosmotic environment preserved their ability to sense and repair newly introduced DNA damage. DNA repair occurred under conditions of both hyperosmolar salt and sorbitol, pointing out that early signals emitted by these solutes are similar and that the osmo-regulatory response of nucleus pulposus cells stems rather from cell volume alterations than from elevated intracellular ionic concentration. When nucleus pulposus intervertebral disc cells were investigated at the transcriptome level using the genome array technology, hundreds of genes were found to be differentially expressed after hyperosmotic treatment. Given that one of the first cellular responses towards extracellular osmotic alterations is the transcriptional regulation of transporting molecules responsible for the influx and efflux of ions or organic osmolytes via the cytoplasmic membrane, the observed differential expression of such genes was further investigated and confirmed by RT-qPCR. In details, seven transporters were found to be up-regulated by high osmolality, while two were found to be down-regulated. Functional analysis was performed in order to determine the physiological role of these molecules in nucleus pulposus cells' osmo-regulatory responses. Understanding disc cells' physiology after taking into consideration the conditions of their *in vivo* physicochemical environment is of great importance for the design of novel therapeutic approaches

O 38

Modulation of pathways underlying distinct cell death mechanisms in two human lung cancer cell lines in response to N-methyl-N-nitrosourea treatment

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Despite concerted research efforts, lung cancer is still the leading cause of cancer death worldwide, therefore, new therapeutic approaches are eagerly needed. Methylating agents constitute a widely used class of anticancer drugs, the effect of which on human non small-cell lung cancer (NSCLC) has not been adequately studied. We thus studied the effect of N-methyl-N-nitrosourea (MNU), a model S_N1 methylating agent, on two human NSCLC cell lines; A549($p53^{wt}$) and H157($p53^{null}$). MNU induced cell death through a distinct mechanism in the above cell lines; we further investigated the differential effect of MNU through a time course gene expression profiling study using DNA microarrays at 24, 48 and 72h of treatment. The number of statistically significant differentiated genes, presenting a minimum of 2 fold alteration in their expression, is 920 for A549 and 545 for H157 cells. In both cell lines, the MNU-induced alterations in gene expression became prominent 48h after treatment. Between the two cell lines, only 89 genes were found in common. GO-based analysis associated gene expression changes with modulation of several biological processes. The most significantly altered processes in H157 cells related to immune/inflammation response, cell cycle, adhesion and RNA binding while in A549 cells, to DNA repair, cell cycle, cytoskeleton organization and apoptosis. Overall, in A549 cells a significant number of p53 downstream genes were up-regulated, suggesting the induction of a p53-dependent apoptotic mechanism. The results were further validated through RT-PCR of selected up- and down-regulated genes showing a good correlation with the microarray data.

Keywords *S_N1 methylating agents, NSCLC, DNA damage response, cell death.*

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O 39

Proteomic studies of cervical cancer cell lines for the discovery of early cervical cancer biomarkers

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Cervical cancer is the second most frequent cancer in women while human papilloma virus (HPV) represents its sole etiologic agent. Despite preventive measures, there is still a clinical need for reliable early cervical cancer biomarkers. Malignant transformation of the cervical epithelium is accompanied by qualitative and quantitative changes in protein expression profiles of the infected cells. In the present study, we undertook a systematic analysis of four cervical cancer cell lines exhibiting unique complementary molecular and phenotypic features, by employing 2D electrophoresis and MALDI-TOF mass spectrometry. The cell lines used were the a) HCK1T, a normal cervical epithelium cell line, b) HeLa, a cervical cancer cell line positive for HPV18, c) SiHa, a cervical cancer cell line positive for HPV16, and d) C-33A, a cervical cancer cell line negative for HPV. We identified 169 proteins (92 upregulated and 77 downregulated), differentially expressed between the normal (HCK1T) and the cancer (HeLa, SiHa, C-33A) cell lines combined. The most significant group of differentially expressed proteins was related to cytoskeletal remodeling. Among these, we further analysed the prominent cytoskeletal protein Cofilin 1. The proteomics for Cofilin-1, were consistently validated by Western blot analysis. In both 2D electrophoresis and WB analysis, Cofilin-1 was significantly upregulated in HeLa and C-33A cells, compared to HCK1T. Interestingly, Cofilin-1 represents an important protein for cell migration regulation and apoptosis induced by oxidants, over-expressed in several highly invasive cancer cell lines. These data provide the impetus for further exploitation and validation of Cofilin-1 as a putative cervical cancer biomarker.

O 40

The KUPNetViz: A biological network viewer for multiple -omics datasets in kidney diseases

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Constant technological advances have allowed scientists in biology to migrate from conventional single-omics to multi-omics experimental approaches, challenging bioinformatics to bridge this multi-tiered information. Ongoing research in renal biology is no exception. The results of large-scale experiments, presenting a wealth of information on kidney disease are scattered across the web. To tackle this problem, we recently presented the Kidney and Urinary Pathway Knowledge Base (KUPKB), a multi-omics data repository for renal diseases. Here, we describe KUPNetViz, a biological graph exploration tool which enables the integration of multi-layered experimental data over different species, renal locations and renal diseases to protein-protein interaction networks and allows association with biological functions, biochemical pathways and other functional elements such as miRNAs. KUPNetViz focuses on the simplicity of its usage and the clarity of resulting networks by reducing and/or automating advanced functionalities present in other biological network visualization packages. In addition, it allows the extrapolation of biomolecule interactions across different species, leading to the formulations of new plausible hypotheses and the suggestion of novel biological mechanisms. We demonstrate the value of KUPNetViz by two usage examples: the integration of calreticulin as a key player in a larger interaction network in renal graft rejection and the novel observation of the strong association of interleukin-6 with polycystic kidney disease. The KUPKB and KUPNetViz are accessible through http://www.kupkb.org.

O 41

Neurosteroidal agonists of NGF receptors: Neuroprotective properties and neurogenic actions

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Neurotrophins control neuronal cell fate and function during development and adulthood. They act through tyrosine kinase Trk and pan-neurotrophin $p75^{NTR}$ receptors, exerting potent neuroprotective and neurogenic effects. Despite the demonstrated beneficial effects, the therapeutic usefulness of neurotrophins is compromised by their polypeptide nature and their restricted penetrance to the blood-brain barrier (BBB). We have recently shown that neurosteroid dehydroepiandrosterone (DHEA) prevents neuronal apoptosis (*Charalampopoulos et al, PNAS 2004*), through binding to TrkA and $p75^{NTR}$ receptors (*Lazaridis et al, PLoS Biol 2011*), activating prosurvival kinases and anti-apoptotic Bcl-2 proteins, preventing thus the apoptotic loss of NGF receptor positive sensory in NGF null mice. However, DHEA is metabolized *in vivo* to sex steroids, affecting the endocrine system. We have recently synthesized 17-spiro analogs of DHEA with anti-apoptotic, neuroprotective properties (IC_{50} at nanomolar levels), deprived of androgenic-estrogenic actions (*Calogeropoulou et al, J Med Chem 2009*). In the present study, we report that synthetic DHEA derivative BNN27 specifically interacts with NGF receptors, TrkA and $p75^{NTR}$ at nanomolar concentrations. BNN27 induced TrkA tyrosine phosphorylation, affecting downstream signaling of Akt and MAPKs in sympathetic neurons and regulated TrkA internalization. Moreover, BNN27 was shown to promote the interaction of $p75^{NTR}$ receptors with its effector factors RhoGDI, RIP2 and TRAF6. It also partially reversed apoptosis of NGF-dependent embryonic sensory neurons of NGF null mice. The neurogenic properties of BNN27 are also tested in 2D and 3D-collagen cultures of embryonic and adult neural stem cells. BNN derivatives may serve as lead molecules to develop BBB permeable, neurotrophin-like small molecules (microneurotrophins) with potential applications in the treatment of neurodegenerative diseases and brain trauma (*Gravanis et al, Science Signaling 2012*).

***Keywords**: Neurotrophins, neurosteroids, neurodegeneration, neural stem cells,*

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O 42

VEGF165 partnership with receptor protein tyrosine phosphatase beta/zeta mediates VEGF165-induced endothelial cell migration

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Vascular endothelial growth factor 165 ($VEGF_{165}$), the dominant isoform of VEGF, induces numerous functions of endothelial cells, such as growth, migration and angiogenesis. These functions are mainly mediated via VEGF receptor type 2 (VEGFR2) and its interaction with co-receptors like $\alpha_v\beta_3$ integrin. Receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) and nucleolin (NCL) are receptors for another heparin-binding growth factor, pleiotrophin (PTN). Binding of PTN to RPTPβ/ζ activates c-Src, which leads to β_3 integrin Tyr773 phosphorylation, and is required for cell surface NCL localization and the subsequent PTN-induced endothelial cell migration. Here it is suggested that $VEGF_{165}$ also binds RPTPβ/ζ. In brief, immunoprecipitation and *in situ* proximity ligation assays showed that $VEGF_{165}$ directly interacts with RPTPβ/ζ in endothelial cells. In addition, RPTPβ/ζ expression regulated $VEGF_{165}$-induced VEGFR2-$\alpha_v\beta_3$ interaction. Immunofluorescence and confocal imaging suggested that RPTPβ/ζ expression and β_3 Tyr773 phosphorylation are involved in $VEGF_{165}$-induced cell surface NCL localization, similarly to the effect of PTN. Western blot analyses and migration assays, moreover, indicated that RPTPβ/ζ expression and β_3 integrin Tyr773 phosphorylation are important for $VEGF_{165}$-induced endothelial cell migration. These results demonstrate the role of RPTPβ/ζ in establishing the interaction between VEGFR2 and $\alpha_v\beta_3$, as well as mediating the translocation of NCL to the cell surface, both events being essential for endothelial cell migration. These data suggest that RPTPβ/ζ being a binding partner of $VEGF_{165}$ may prove a suitable candidate for the development of additive or alternative anti-VEGF therapies.

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O 43

The cadherin topology in endothelial cells is influenced by plasma membrane microdomains

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Endothelial adherens junctions consist of the transmembrane vascular endothelial cadherin (VE-cadherin) associated with cytoplasmic catenins. We found that VE-cadherin is located in special lipid microdomains of the plasma membrane and it colocalizes to a certain degree with PIP2 at the plasma membrane, shown by microscopy and biochemical assays. We constructed several VE-cadherin mutants, coding either for the entire transmembrane and the cytoplasmic domains or others lacking certain cytoplasmic domains linked to specific cadherin functions: a) domains known to be involved in cadherin-actin associations and b) a novel domain found to influence VE-cadherin topology at the plasma membrane. When the cytoplasmic domains of VE-cadherin was compared with other classic cadherin molecules was found that it displays a unique positively charged domain of 23 aminoacids located between the transmembrane and the p120 catenin binding domains. When deleted, the expressed VE-cadherin mutant failed to colocalize with PIP2. We conclude that in endothelial cells the topology of VE-cadherin is based on a "dual affinity" towards both the cytoskeleton and the membrane lipid microenvironment.

O 44

Interactions of Opioid Receptors with Regulators of G Protein Signalling

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Opioid receptors (OR) μ, δ, and κ couple to Gi/Go proteins to modulate a variety of physiological responses in the nervous system through activation of a diverse array of effector systems. Apart from G proteins, opioid receptor activity is also controlled by interactions with other proteins which contribute to the intricate fine tuning opioid receptor signalling (Georgoussi et al. 2012, Leontiadis et al., 2009). Regulators of G protein Signalling (RGS) comprise a large multifunctional protein family that accelerate GTP hydrolysis of Gα subunits and modulate G protein coupled receptor (GPCR) signalling. Pulldown experiments using GST fusion peptides encompassing intracellular portions of opioid receptors demonstrate the ability of two members of the B/R4-RGS family, such as RGS4 and RGS2, to interact directly with all three OR subtypes. Co-immunoprecipitation studies showed that both RGS proteins confer selectivity to the opioid receptors for coupling with a specific subset of G proteins. On the other hand, using a series of functional assays we demonstrate that although both RGS members co-localize with the membrane bound receptor upon agonist stimulation, they differentially alter OR mediated adenylyl cyclase activity while displaying a similar effect on ERK1,2 phosphorylation. Measurements of cell surface receptors in HEK293 cells co-expressing the ORs along with RGS members have shown that RGS4 and not RGS2 influence the internalization fate of δ-OR. Collectively, our results demonstrate that RGS2 and RGS4 are novel interacting partners and pharmacological targets that negatively modulate opioid receptor signaling.

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O 45

Tryptase in human nasal polyps tissue is able to degrade the insulin-like growth factor binding protein-3 (IGFBP-3)

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Nasal polyposis is a chronic inflammatory disease of the nasal mucosa, that characterized by inflammatory cell infiltration, modifications of epithelial differentiation, tissue remodelling, extracellular matrix accumulation, and oedema.

Significant IGFBP-3-degrading activity, was detected in extracts of nasal polyps tissues, which inhibited by benzamidine hydrochloride. This enzymatic activity, purified by affinity chromatography, was able to cleave the IGFBP-3 in two major fragments of molecular mass 27 and 14kDa, which were not bound to IGF-I. Upon SDS-PAGE of affinity purified enzymatic activity, a protein band of molecular mass ~27 kDa appeared, which was found by MALDI-TOF mass spectrometry to contain peptides identical to human tryptase. Immunohistochemistry analysis revealed that a significant population of masts cells occurs in polyps tissues, and tryptase activity was detected in all polyps tissue extracts tested. When the polyps affinity purified enzyme was tested in parallel with purified tryptase from human lung, it was found that both enzymes are able to cleave the IGFBP-3 in identical manner, either free or in complex with IGF-I, in fragments that do not bind IGF-I, and they are inhibited by tryptase specific inhibitor APC. In addition, the IGFBP-3 that significantly suppressed the IGF-I-induced proliferation of nasal polyp fibroblasts, upon treatment with both enzymes lost its suppressive activity.

In conclusion, the tryptase produced from mast cells in nasal polyps tissue may be responsible for the in situ release of IGF-I from IGF-I/IGFBP-3 complexes, which subsequently may stimulate the growth of both epithelium and blood vessels in the sinuses, contributing into polyposis pathogenesis.

O 46

Dynamic interplay between normal endothelium and breast cancer cells via altered expression of matrix molecules

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Metastasis involves the adhesion to endothelium of blood/lymph vessels and transmigration through it. The aim of the present study was to determine the cellular responses that breast cancer cells (BCC) (MDA-MB-231 & MCF-7), elicit in the human umbilical vein endothelial cells (HUVEC). For this purpose two models were utilized;one involves HUVEC culture in the presence of BCC-derived conditioned media (CM) and the other co-culture of both cell populations in a Transwell system. We found that CM from BBCs decreases HUVEC cells migration, affecting also cytoskeleton organization, whereas the adhesion of cancer cells is favored by the presence of HUVEC-secreted matrix effectors. Real-Time PCR analysis showed that HA receptor CD44 and HA synthase HAS2 gene expressions in HUVEC are substantially up-regulated, in accordance with elevated HA levels. BBCs take advantage of hyaluronan-rich matrices to invade through ECM coated transwell. ICAM-1 and VCAM-1, key adhesion molecules in trafficking of CC across endothelial and epithelial barriers, are upregulated. Notably, the expression of MT1-MMP, MMP-2 by HUVEC is significantly downregulated in both culture systems, where MMP-9 is up-regulated. On the other hand, ubiquitin proteasome system is the central protein degradation mechanism. Gene expression and activity of β5 proteasomal subunit is upregulated, especially by the action of MDA-MB-231 cells on HUVEC. Conclusively, these data suggest that factors secreted by BCC regulate the expression of matrix macromolecules, implicated in endothelial functional properties as well as proteasomal protein degradation.

O 47

A complex signaling network involving casein kinase 2 is required for Hepatitis C Virus (HCV) core protein-mediated modulation of the iron-regulatory hepcidin gene expression

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HCV infection causes severe liver disease and hepatocellular carcinoma. It is associated with hepatic iron overload and hyperferremia that correlate to poor antiviral responses. Hepcidin (HAMP) is synthesised by the liver and controls iron homeostasis by inducing degradation of the cellular iron exporter ferroportin. Hepcidin is increased by inflammation and iron excess. Chronic HCV patients possess low hepcidin levels, nevertheless elevated HAMP mRNA has been reported in HCV core transgenic mice and HCV replicon-expressing cells. We aimed to investigate the effect of HCV core protein on HAMP gene expression and delineate the complex interplay of molecular mechanisms involved. We found that HCV core protein up-regulated HAMP promoter activity, mRNA and secreted protein levels. HCV core conferred enhanced promoter activity through adjacent BMP and STAT binding sites in the proximal HAMP gene promoter. Dominant negative constructs and pharmacological inhibitors against STAT3 and SMAD4 confirmed the participation of both pathways in HCV core –mediated hepcidin regulation. Both STAT3 and SMAD4 expression levels were elevated in the presence of HCV core, which orchestrated SMAD4 translocation into the nucleus and STAT3 phosphorylation. Importantly, we provide novel evidence that the multifunctional JAK/STAT-activating casein kinase 2 (CK2) acted in synergy with HCV core to significantly enhance HAMP gene expression through CK2-driven activation of both STAT3 and SMAD/BMP pathways. We also showed that CK2 expression and kinase activity were elevated by HCV core. Consequently, HCV core up-regulates HAMP gene transcription via a complex signalling network that requires both SMAD/BMP and STAT3 pathways and CK2 involvement.

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O 48

The Role of Smyd3 in Hepatocellular Carcinoma and Colorectal Cancer

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Altered expression or activity of histone lysine methylases and demethylases in cancer lead to aberrant chromatin modification patterns, which contribute to uncontrolled cell proliferation via cancer-specific deregulation of gene expression programs. Smyd3 is a histone lysine methylase catalyzing H3K4 and H4K5 methylation. Smyd3 expression is upregulated in human colorectal (CRC) and hepatocellular carcinomas (HCC). Previous studies conducted in cultured cell lines suggested that Smyd3 might be involved in the regulation of cell proliferation. To study the role of Smyd3 in vivo, we generated knock-out mice using ES cells harboring gene-trapped allele of Smyd3.

We analyzed the effects of Smyd3 deficiency in chemically induced models for HCC and CRC. Our results indicate that Smyd3 is required for the development and progression of HCC and CRC as lack of Smyd3 significantly reduced tumor incidence and tumor load in the livers of DEN-treated or colons of DMH/DSS-treated mice. Smyd3-deficiency affected both, the rate of proliferation and oncogenic transformation of hepatocytes and epithelial cells in the colon. In addition we demonstrate that Smyd3 regulates Epithelial Mesenchymal Transition (EMT) via modulating MMP (***Matrix MetalloProteinases***) expression in the chemically induced liver and colon cancer models.

O 49

Geminin binds onto distal and proximal elements of HoxA9 and regulates fate commitment decisions of embryonic haemopoietic stem cells

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The hematopoietic system is generated by a hematopoietic stem cell (HSC) population that maintains its multipotency and self-renewal capacity throughout. During hematopoiesis, mechanisms that control self-renewal, fate commitment and differentiation decisions should be precisely balanced in order to prevent exhaustion of fetal and adult HSCs and maintain lifelong generation and replacement of blood cells and response to hematological stress and challenges.

Our findings show that Geminin is essential for the generation of the hematopoietic system. Conditional inactivation of mouse Geminin resulted in accumulation of HSCs and lack of fate-committed and differentiated blood cells. Transcriptomics analysis of Lin- cells that lack Geminin expression showed upregulation of genes associated with "stemness". Moreover, depletion of Geminin in K562 cells lead to overexpression of HoxA9, a homeobox gene that is generally silenced in K562 cells, similarly to what it was observed in HSCs in the absence of Geminin. Chromatin immunoprecipitation assays showed that upon Geminin silencing, repressive epigenetic marks were almost depleted from promoter proximal loci with the concomitant enrichment of epigenetic marks associated with transcriptionally active genes. Furthermore, using two different antibodies we showed that Geminin binds to both distal (enhancer-like) and proximal regulatory loci of the HoxA9 genomic locus.

Our results suggest that Geminin binds directly and regulates HoxA9 transcriptional repression. This regulation that might be a key event in the maintenance of balance between self-renewing HSCs and fate restricted haemopoietic progenitor cells.

O 50

The role of nanoscale roughness of polymeric substrates on cell adhesion and proliferation

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Surface nanotopography influences cells attachment and proliferation, as well as other basic cell functions. The aim of this work was to study the effect of surface nanotexturing on the adhesion, viability and proliferation of normal fibroblasts and cancer cells. Thin poly(methyl methacrylate) (PMMA) films on Si substrate were treated with O_2 plasma under different etching conditions (bias voltage: 0-100 Volts; electrode temperature: 15 ^oC and 65 ^oC; etching time: 3 s, 1 min and 3 min) in order to achieve varying roughness and surface nanotexturing[1]. The O_2 plasma nanotextured surfaces along with untreated ones were used as substrates to culture normal fibroblasts and cancer cells (fibrosarcoma cell line HT1080). It was found that normal fibroblasts adhered and proliferated on the untreated PMMA surfaces while HT1080 adhered negligibly on these surfaces. On the other hand, employing O_2 plasma nanotextured surfaces prepared using bias voltage of -100 V for 3 min as culture substrates, three times more HT1080 cells adhered after one day culture compared to normal cells. Furthermore, at three days, the number of HT1080 cells was increased 6 times compared to the one day culture, whereas the number of normal cells was reduced by half providing a ratio of cancer to normal cells of approximately 33. In conclusion, O_2 plasma nanotextured PMMA surfaces could be a useful tool for the enrichment and isolation of cancer cells derived from tissues suspected for neoplasias and thus, could help to improve cancer diagnosis and facilitate personalized therapy approaches.

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O 51

Intracellular mediators of cortical interneuron development

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Impaired interneuron function in the cortex results in neurodevelopmental disorders (schizophrenia, epilepsy, autism). Extracellular signals that guide the migration of interneurons to the cortex to form appropriate synapses have been analyzed. However, intracellular components involved in interneuron development are still unknown. Rac-proteins are RhoGTPases that integrate multiple extracellular signals required for essential processes in diverse cell types as cytoskeleton organization, vesicle trafficking, transcription, cell cycle progression, and apoptosis. Our work examines the role of Rac1 in interneurons specifically derived from the medial ganglionic eminence (MGE), a population comprising the majority of cortical interneurons.

We used Cre/loxP technology to uncover a cell autonomous and stage-specific requirement for Rac1 activity within proliferating interneurons for the co-ordination of cell cycle progression with differentiation and migration (Vidaki et al., 2012). Most mice die after 4 weeks due to epileptic seizures since 50% of GABAergic interneurons are absent from the postnatal cortex. We found that Rac1 is necessary for the transition from G1 to S phase, in part by regulating CyclinD levels and Rb-phosphorylation.

In addition, MGE cells *in vitro* show cytoskeletal alterations such as a significant reduction of the leading process length and in growth cone formation in the absence of Rac1 protein. Our observations indicate that the absence of Rac1 could affect the turnover of F-actin resulting in improper formation of leading processes and axon growth cone formation. Our aim is to decipher the molecular mechanisms underlying the observed defects in mice lacking Rac1 from their cortical interneurons.

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O 52

A novel mechanism of cardioprotection through TNF-α-mediated alternative cytoskeleton formation

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The adult myocardium demonstrates a unique system of adaptation upon stress stimuli, in an effort to maintain its overall homeostasis. This compensatory mechanism remains a mystery. Tumor Necrosis Factor-α (TNF-α) is one of the major - stress-induced - pro-inflammatory cytokines that is upregulated in end-stage heart failure. Its sustained expression is considered detrimental for the heart while its potential cardioprotective mechanisms remain elusive. Here we propose a novel cardioprotective function for TNF-α overexpression in a genetic heart failure model, through ectopic expression of the simple epithelium-specific Intermediate Filament (IF) proteins Keratin-8 and Keratin-18 (K8/18), in mice lacking the desmin IF network (TNFαDes-/-). Specifically, we show complete amelioration of the extensive myocardial degeneration, inflammation and replacement fibrosis and a remarkable improvement of cardiac function. The *de novo* K8/18 expression is cardiomyocyte-specific and K8/18 form a cytoskeletal network which localizes mainly at the Intercalated Discs (IDs). This alternative K8/18 cytoskeleton confers cardioprotection by a mechanism that maintains ID and mitochondrial integrity. Importantly, we discovered that in the cardiomyocytes of human failing myocardium, where TNF-α is induced, K8/18 network is also ectopically expressed and localizes primarily at IDs where desmin is prominently absent. This is the first report to propose a TNFα-mediated cardiac expression of K8/18 IF proteins, which act as stress-induced cardioprotective factors in the failing heart where they compensate for the defective cytoskeleton, a phenomenon of major clinical significance as it expands globally to human heart failure.

Supported by *"Heracleitus II" scholarship by the European Union and Greek national funds to SP and by Greek Secretariat of Research and Development grant ESPA SYNERGASIA SYN 965 to YC*

O 53

Functional Interactions between Mouse Double Minute 2 Protein and a putative oxidase

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We have isolated the putative oxidase p110b, which is encoded by the oncogene ZNF217, a component of a HelaS-derived HDAC1 complex, as a novel MDM2-interacting protein using MDM2-specific affinity chromatography and mass spectrometry. Specifically, p110b interacts with the amino terminal part of MDM2, which also contains at least two lysines that are possible methylation targets. P110b contains two FAD binding domains, it can interact *in vivo* with MDM2 and can inhibit the p300-mediated acetylation of p53. P110b also can repress the ability of p53 to activate the p21 promoter either via a p110b consensus binding site on the p21 promoter or through complex formation with HDAC.methylase enzymes . Over-expression of p110b reduces the steady-state levels of acetylated p53 in cell lines and can inhibit the p300-mediated acetylation of p53. These findings suggest that MDM2 controls p53 acetylation levels, and hence its function, by non-ubiquitination-dependent p110b/ HDAC1/2 -mediated interactions. We have preliminary computational and experimental evidence that methylation/demethylation pathways might be linking MDM2E, an 3 ubiquitin ligase, to transcription and gene expression via novel mechanisms.

O 54

Novel osteoporosis models by overexpressing human RANKL in transgenic mice

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Receptor activator of nuclear factor-κB ligand (RANKL) is a central regulator of bone remodeling by mediating osteoclast-induced bone resorption. Overproduction of RANKL is implicated in a variety of degenerative bone diseases such as osteoporosis and its specific inhibition effectively reduces the incidence of osteoporotic fractures. We have recently generated transgenic mice overexpressing human RANKL (TghuRANKL) in order to model RANKL-mediated pathologies. To achieve a correct pattern of human RANKL expression in the mouse, a 200kb genomic fragment containing the whole human RANKL gene was used as a transgene. Quantitative bone analysis revealed a mild phenotype in the low copy number Tg5516 line displaying trabecular bone loss and reduced biomechanical properties at 3 months of age. A more severe osteoporotic phenotype was identified in the high copy Tg5519 line developing trabecular bone loss, severe cortical bone porosity, and decreased bone strength by the age of 3 months. The numbers of osteoclasts at the cortical surfaces as well as serum markers of bone turnover were significantly increased in Tg5519 mice. The observed phenotypes developed in both sexes, whereas the levels of human RANKL expression were correlated with disease severity. Interestingly, treatment of TghuRANKL mice with known anti-resorptive drugs effectively inhibited bone resorption proving the significance of such mice in preclinical evaluation studies of novel anti-osteoporotic compounds. These novel human RANKL transgenic models of osteoporosis represent a unique tool for understanding the pathogenic mechanisms in bone resorption as well as for the preclinical evaluation of novel inhibitors that target human RANKL and osteoclasts.

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O 55

Reconstituted high density lipoprotein (rHDL) modulates T effector cell function in a murine model of rheumatoid arthritis

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High density lipoprotein (HDL) is a negative risk factor for Cardiovascular Disease due to its many atheroprotective functions. HDL functions are abrogated in patients with rheumatoid arthritis. Recent evidence suggests a role of HDL in modulating both innate and adaptive immune responses. We sought to investigate the role of HDL during the development of an autoimmune response in a mouse model of antigen-induced arthritis. C57BL/6 mice were subcutaneously immunized with ovalbumin (OVA) and the draining lymph nodes (LN) were excised and cultured in the presence of varying concentrations of reconstituted HDL containing apoA-I (rHDL-AI) in the presence or absence of the antigen. OVA-primed LN cells secreted increased levels of IFN-γ and IL-17 that were significantly suppressed in the presence of rHDL-AI in a dose-dependent manner. rHDL-AI was found to exert a suppressive effect on T cell proliferation. We also assessed the effect of rHDL-AI on bone marrow-derived dendritic cell (BM-DC) activation and maturation. We found that rHDL-AI treatment of LPS-stimulated BM-DCs suppressed the secretion of IL-6 and IL-8 and the expression of ABCA1 and ABCG1 transporters, two major players of the cholesterol efflux pathway. Finally, rHDL-AI treatment induced phenotypic differences on cell surface molecules of LPS-stimulated BM-DCs such as CD40, CD86 and PDL-1. In conclusion, rHDL-AI exerts a direct immunomodulatory function on T cells *in vitro* by suppressing their proliferation and the expression of inflammatory cytokines. These data identify HDL as an important player in the homeostatic regulation of inflammatory responses and a potential therapeutic target for chronic inflammatory diseases.

O 56

Novel insights into the properties and fate of naturally secreted α-synuclein

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Recent evidence suggests that extracellular α-synuclein strains are implicated in the progression of Parkinson's disease (PD) pathology. However, most of these studies have employed recombinant α-synuclein. Deregulation in the normal degradation and clearance of naturally secreted α-synuclein could underlie some or many cases of the disease. To date, the degradation mechanisms involved have received very little attention. We show for the first time that naturally secreted α-synuclein forms are resistant to direct proteolysis by kallikrein-related peptidase 6 (KLK6), an enzyme known to cleave recombinant α-synuclein. This differential susceptibility appears to be partially due to the non-covalent association of secreted α-synuclein with lipids. We further provide evidence of a novel proteolytic cascade that regulates secreted α-synuclein, mediated by both KLK6 and a secreted metalloprotease activity. Our results suggest that physiologic modifications affect the biochemical behavior of secreted α-synuclein and further provide novel insights into mechanisms and potential targets for therapeutic interventions.

O 57

Contribution of germline CHEK2 mutations in BRCA1 and BRCA2 - negative breast cancer patients in Greece

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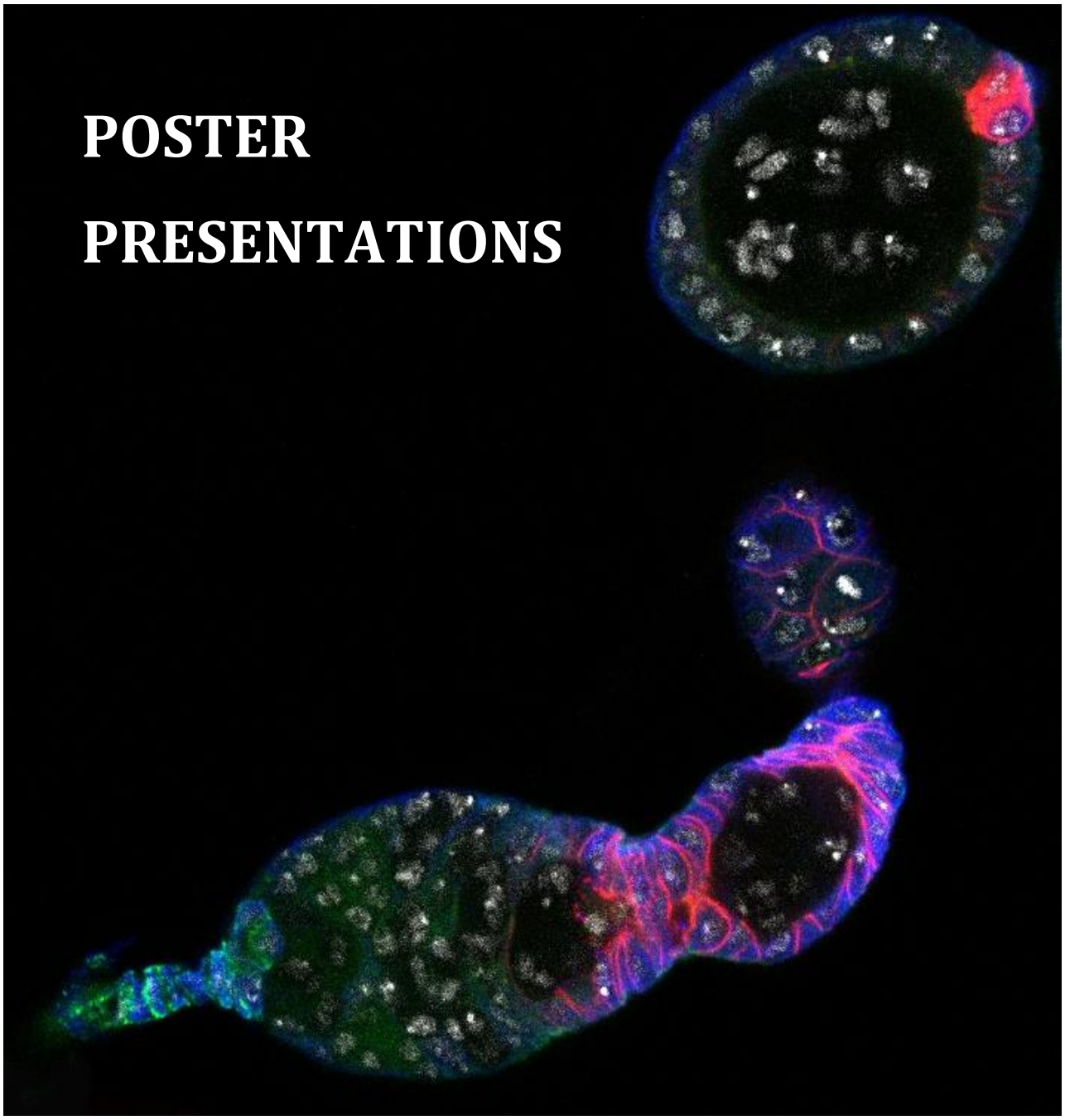
CHEK2 gene encodes a protein kinase that has a central role in controlling cell-cycle checkpoints after DNA damage and stalled replication. *CHEK2* is normally activated in response to DNA double-stranded breaks, regulates the function of BRCA1 protein in DNA repair by homologous recombination and maintain the genomic integrity. Germline *CHEK2* mutations are associated with increased risk of breast cancer in women and other malignancies. To elucidate the role of *CHEK2* as a cancer susceptibility gene in breast cancer patients in Greece we genotyped 428 females diagnosed with synchronous or metachronous breast cancer or having at least one family relative diagnosed with cancer, 206 females diagnosed with early onset breast cancer (<45 years) without a reported family history and 445 healthy age-matched females. All patients had previously been tested negative for Greek *BRCA1* founder and recurrent mutations. Missense and loss-of-function mutations were found in 15 of 428 (3.5%) patients with family history, while ten missense mutations with possible deleterious effect were detected in 206 (4.85%) early onset breast cancer patients without family history. Mean age of breast cancer diagnosis was 42.2 and 34.7 years, respectively. Eleven missense and nonsense mutations (2.7%) were detected in healthy women. This can be explained by the moderate cancer susceptibility of *CHEK2* gene. The present data provide the first evidence for the existence of a large proportion of *CHEK2* mutations in breast cancers in Greece. Women with early-onset breast and/or family history should be considered for *CHEK2* mutation testing, after a negative *BRCA1* and *BRCA2* result.

POSTER PRESENTATIONS



P1

Delineating the role of desmin in cardiac differentiation and trans-differentiation

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Heart disease is the leading cause of mortality in the Western world. Given the limited regenerative capacity of the heart and the insufficient therapeutic approaches, much interest has been focused on cardiac regenerative medicine. Important steps towards possible therapeutic generation of cardiomyocytes were achieved with the direct reprogramming, or transdifferentiation, of fibroblasts into functional cardiomyocytes through ectopic expression of three transcription factors GATA4, Mef2C and Tbx5 (GMT), however with rather low efficiency. Here, we propose that desmin, a muscle specific protein that has been studied mostly for its contribution to function, stability and longevity of the working myocardium, plays a pivotal role in reprogramming. We investigate the ability of fibroblasts to transdifferentiate into cardiomyocytes upon GMT expression in the presence or absence of desmin. Our initial observations reconfirm that desmin, a so long thought structural protein, is indeed important for cardiac cellular commitment. Our studies will examine, at the molecular and cellular level, the mechanism by which desmin controls the balance between proliferation and differentiation, potentially providing new avenues for the development of targeted therapeutic approaches for cardiac diseases.

P2

Mitochondrial dynamics as a key determinant of longevity in Drosophila melanogaster

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Cellular homeostasis and mitostasis (homeostasis of mitochondria) are, among others, regulated by a delicate balance between mitochondrial fusion and fission. These processes depend on the coordinated function of four conserved (from yeast to mammalian) GTPases, namely the mitofusins Mfn1 and Mfn2 along with Optic Atrophy 1 (OPA1) which are implicated in mitochondrial fusion, as well as dynamin-related protein 1 (Drp1) that regulates fission. Both mitochondria fusion and fission are central to the modulation of cellular homeostasis as they control mitochondria biogenesis and mitophagy. The disruption of mitostasis has been implicated in aging and neurodegenerative diseases, while recent studies have indicated that the ubiquitin-proteasome system is implicated in the regulation of mitochondrial fusion. Herein, we present our preliminary analyses regarding the implication of mitochondrial dynamics in the molecular processes of ageing and their links to the main cellular proteolytic pathways, namely the ubiquitin-proteasome and the autophagy-lysosome systems. Our *in vivo* studies at the model organism *Drosophila melanogaster* have revealed that impairment of mitochondrial fusion decreases the muscle strength of flies and significantly accelerates ageing. Moreover, disruption of mitochondrial fusion processes impacts on the catalytic activities and the expression levels of the proteasome, as well as on the enzymatic activities of key lysosomal enzymes, indicating the existence of a circuit of sensors that signal disruption of mitostasis to proteolytic machineries. Future experiments in our lab aim to clarify the molecular basis of these findings.

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P3

Neonatal fibroblasts and living cell constructs exert paracrine anti-fibrotic effects on young and senescent adult human skin fibroblasts

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Increased numbers of senescent cells observed in chronic wounds may affect wound healing either due to their inability to proliferate, or by altering the local microenvironment. Living cell constructs (LCCs) containing fibroblasts and keratinocytes have been used as therapeutic approaches against chronic wounds. Accordingly, aim of the present work was to examine the effects of factors secreted by early passage neonatal fibroblasts and LCCs – in the form of conditioned media (CMs) – on senescent vs. early passage adult skin fibroblasts.

Target cells were fibroblasts senescent either due to subsequent divisions (replicative senescence; RS) or due to an exogenous stress (stress-induced premature senescence; SIPS). Fibroblast functions related to the healing process, i.e. cell proliferation, collagen synthesis, metalloproteinase and alpha-smooth muscle actin (α-SMA) expression were studied following CM treatment.

All CMs induced early passage fibroblast proliferation – while having no effect on the proliferation of senescent fibroblasts, as expected – and inhibited overall collagen synthesis both in early passage and in senescent fibroblasts. The LCC-derived CM was found to be more potent than fibroblast-derived CMs and, furthermore, to inhibit α-SMA expression.

In conclusion, the above results may indicate anti-contractile and anti-fibrotic activities of factor(s) secreted by neonatal skin fibroblasts, and more intensely by LCCs. Furthermore, although the senescent cells that are present in the chronic wound environment possess a catabolic phenotype, they seem to respond to paracrine factors secreted by early passage cells present in LCCs.

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P4

Specific lipofuscin staining as a novel biomarker to detect replicative and stress-induced senescence
A method applicable in cryo-preserved and archival tissues

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**The authors contributed equally to this work*

There is shortage of extensive clinicopathologic studies of cellular senescence because the most reliable senescence biomarker, the detection of Senescence-Associated-beta-galactosidase activity (SA-β-gal), is inapplicable in archival material and requires snap-frozen tissues. We validated the histochemical Sudan-Black-B (SBB) specific stain of lipofuscin, an aggregate of oxidized proteins, lipids and metals, known to accumulate in aged tissues, as an additional reliable approach to detect senescent cells independently of sample preparation. We analyzed cellular systems in which senescence was triggered by replicative exhaustion or stressful stimuli, conditional knock-in mice producing precancerous lesions exhibiting senescence, and human preneoplastic lesions known to contain senescent cells. In the above settings we demonstrated co-localization of lipofuscin and SA-β-gal in senescent cells *in vitro* and *in vivo* (cryo-preserved tissue), strongly supporting the candidacy of lipofuscin for a biomarker of cellular senescence. Furthermore, cryo-preserved tissues positive for SA-β-gal were formalin-fixed, paraffin-embedded, and stained with SBB. The corresponding SA-β-gal positive tissue areas stained specifically for lipofuscin by SBB, whereas tissues negative for SA-β-gal were lipofuscin negative, validating the sensitivity and specificity of the SBB staining to visualize senescent cells in archival material. The latter unique property of SBB could be exploited in research on widely available retrospective tissue material.

P5

Senescent human periodontal ligament fibroblasts after replicative exhaustion or ionizing radiation have a decreased capacity towards osteoblastic differentiation

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*: Dimitrios Konstantonis and Adamantia Papadopoulou contributed equally to this work.

Loss of teeth increases with age or after genotoxic treatments, like head and neck radiotherapy, due to periodontium breakdown. Periodontal ligament fibroblasts represent the main cell type in this tissue and are crucial for the maintenance of homeodynamics and for its regeneration. Here, we have studied the characteristics of human periodontal ligament fibroblasts (hPDLF) that became senescent after replicative exhaustion or after exposure to ionizing radiation, as well as their ability for osteoblastic differentiation. We found that senescent hPDLF express classical markers of senescence, as well as a catabolic phenotype, as shown by the decrease in collagen type I and the increase of MMP-2 expression. In addition, we observed a considerably decreased expression of the major transcription factor for osteoblastic differentiation, i.e. Runx2, a down-regulation which was found to be p53-dependent. In accordance to the above, senescent cells have a significantly decreased Alkaline Phopshatase gene expression and activity, as well as a reduced ability for osteoblastic differentiation, as found by Alizarin Red staining. Interestingly, cells from both type of senescence express similar characteristics, implying analogous functions in vivo. In conclusion, senescent hPDLF express a catabolic phenotype and express a significantly decreased ability towards an osteoblastic differentiation, thus probably affecting tissue development and integrity.

P6

Study of the effects of a natural compound in the lifespan and healthspan of the nematode Caenorhabditis elegans

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Senescence is the process of cumulative changes and damage to molecular and cellular structure that disrupts metabolism with the passage of time, resulting in homeostasis collapse and death. Senescence occurs both on the organismal level as well as on the level of its individual cells. Some of the changes observed are related to the increase of oxidative stress and the reduced function of the basic cellular proteolytic mechanism, the proteasome. Proteasomes are protein complexes responsible for the degradation of normal or damaged proteins. Therefore, proteasomes are part of a major mechanism by which cells regulate the concentration and therefore the function of particular proteins and degrade misfolded or otherwise damaged proteins. The objective of the present work is the study of the effects of natural compounds on the lifespan and healthspan of the nematode *Caenorhabditis elegans*, which is used as a model organism for aging. Main direction of the study is the investigation of the effects of these compounds on the functionality of the proteasome and other signaling pathways associated with the aging process (e.g. dietary restriction signaling pathway, insulin/IGF-1-like signaling pathway), by conducting lifespan assays in wild type and several mutant strains, protein activities assay, immunoblot analysis and Real-time PCR analysis, in order to identify anti-aging compounds with possible proteasome activating properties. These results will show us, for the first time, if the activation of the proteasome in a multicellular organism is achievable through natural compounds that exist in the Greek flora and are part of the Mediterranean diet.

P7

Study of the role of the proteasome in senescence of stem cells

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Ageing can be defined as a multifactorial process leading to a gradual decline of self-defensive mechanisms, reduced regenerative capacity of all tissues and organs and an exponential accumulation of damage at the molecular, cellular and organismal level. Stem cells are responsible for tissue renewal and loss of their stemness may contribute to tissue aging. The proteasome is the main cellular proteolytic system that plays a fundamental role in maintenance of cellular homeostasis. Proteasome alterations are associated with various biological phenomena including cellular senescence and ageing in different cell types and tissues. However, little is known regarding the role of the proteasome and other antioxidant responses in senescence of stem cells. In order to shed light on the limited data on stem cell ageing, we employed both Wharton-jelly and adipose derived adult mesenchymal stem cells. We analysed all three proteasomal peptidase activities (chymotrypsin-like, trypsin-like and caspase-like) and characterized the RNA and protein expression levels of representative subunits. Additionally we evaluated the levels of oxidatively modified proteins in early, middle and late passage cells. Analysis of antioxidant defense mechanisms will open the road to innovative stem cell-based interventions to improve the quality of human life in old age ('healthspan'), including treatment of late-onset diseases

P8

The effects of Bisphenol-A on the development and diapause of Sesamia nonagrioides

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The monomer bisphenol-A (BPA, 2,2-bis-(4-hydroxyphenyl)-propane) is one of the industrial compounds that have generated concerns due to their high production and widespread use in many consumer products. BPA is a xenoestrogen that, potentially, can have adverse effects on humans as well as on wildlife. Exposure to Bisphenol-A (BPA) has been reported to affect growth and development in a wide variety of species, although the mechanisms underlying BPA-induced effects are not completely understood. In this study, we tested the effects of three concentrations of BPA (0.1 mg/L, 1 mg/L or 10 mg/L) on the development and diapause of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). In addition we measured the level of alterations caused by the endocrine activity of BPA in *S. nonagrioides*. The ability of bisphenol-A to interfere with two ecdysteroid-dependent physiological processes, molting and development, was evaluated. Bisphenol-A, elicited ecdysteroidal activity as it was indicated by its impact in the prolongation of the intermolt period and its interference with the developmental processes. In addition alterations in larval weight during diapause, indicate the serious environmental effects of bisphenol-A on wildlife

P9

The role of chaperone mediated autophagy in nervous system development

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Proteolytic mechanisms that remove unwanted proteins are of paramount importance for cellular physiology. Among them, chaperone mediated autophagy (CMA) plays a critical role for the clearance of select cellular proteins and has been associated with the degradation of disease-related proteins, such as alpha-synuclein, in the context of neurodegenerative diseases. However, almost nothing is known about the role of CMA in nervous system development. In particular, the aim of this study was to examine the possible involvement of CMA in the early stages of neural differentiation during development. Accordingly, here we present functional data indicating that CMA is critically involved in the differentiation decisions of neural stem cells (NSCs). Specifically, we show that CMA is highly active in NSCs and that Lamp2a and Hsc70, basic components of the CMA machinery, are strongly expressed in *ex vivo* cultured NSCs. Interestingly, Lamp2a, which is the lysosomal receptor and rate limiting step for CMA, is preferentially expressed in early post mitotic neurons as compared to nascent astroglial cells. Most importantly, Lamp2a reduction, by lentiviral-mediated shRNA, strongly impairs the ability of NSCs to produce neurons and induces their potential to generate astrocytes. These data suggest a key role for Lamp2a and consequently of CMA in the regulation of neuronal fate acquisition. To further understand the *in vivo* role of CMA in brain development, we are currently undertaking gain-and-loss of function studies for Lamp2a in the rodent brain. Moreover, we are engaged in detailed proteomic analysis to identify CMA protein-targets that mediate these effects in NSCs

P10

The role of oxidative stress on the proliferation and senescence of nucleus pulposus intervertebral disc cells

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During degeneration and aging, intervertebral discs undergo substantial changes promoted by the overexpression of metalloproteases and cytokines in disc cells. Moreover, herniated and senescent discs are characterized by the accumulation of oxidized proteins. Given that oxidative stress can induce premature senescence in various tissues, aim of this work was to study the effect of hydrogen peroxide (H_2O_2) on the proliferation and induction of premature senescence in primary cultures of human intervertebral disc cells. H_2O_2 rapidly increased the intracellular levels of nucleus pulposus cells' reactive oxygen species (ROS) in a dose-dependent manner, but was significantly cytotoxic only in concentrations ≥ 500 μM. Oxidative stress negatively affected the proliferation rate of the cells and activated all members of the MAPK superfamily, as well as Akt. In addition, H_2O_2 provoked DNA damage to the nuclei of intervertebral disc cells, as shown by the phosphorylation of H2A.X, induced a DNA repair response involving the phosphorylation of ATM and Chk2 and activated the p53-$p21^{WAF1}$-pRb axis. Finally, a transient translocation of the transcription factors NF-kB and Nrf2 form the cytoplasm to the nucleus was observed in H_2O_2–treated cells. Serial exposures to H_2O_2 resulted in the induction of premature senescence, as demonstrated by a decreased ability of BrdU incorporation and an increased percentage of SA-β Gal-positive cells. This senescent phenotype was further verified by the up-regulation of known molecular markers of cellular senescence. Pre-treatment of nucleus pulposus intervertebral disc cells with the scavenger N-acetyl-L-cysteine or the antioxidant molecule glutathione prevented H_2O_2-induced premature senescence.

P11

Transit effect of Geminin absence in adult neural stem cells

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Neural stem cells (NSCs) are self-renewing, multipotent cells residing in the CNS, during embryogenesis and adulthood. In the adult brain, NSCs are located in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus. Our aim is to investigate mechanisms that control the self-renewal and differentiation decisions of the adult NSCs. We have previously shown that in the absence of Geminin, early cortical progenitors exhibit altered cell cycle kinetics and a preference toward self-renewing divisions. Conversely, Geminin overexpression resulted in increased cell cycle exit and neuronal differentiation of embryonic cortical progenitor cells. In order to further elucidate the *in vivo* role of Geminin in the adult NSCs, we generated mice lacking Geminin expression specifically in the adult NSCs. Our data show that NSCs produce the same number of neurons upon Geminin deletion, but with different kinetics. Shortly after the deletion of Geminin, transit-amplifying cells are reduced, even though the total number of proliferating cells is increased. However, at later stages, the total number of adult NSCs is not physiologically affected. Our data suggest that Geminin has a transit role during adult neurogenesis, possibly because mechanisms maintaining the brain homeostasis and size can counter balance for the absence of Geminin.

P12 Pharmacological activation of Nrf2 mediates anti-ageing effects in Drosophila

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Organismal ageing is a complex molecular process driven by diverse molecular pathways and biochemical events. It has been defined as the time-dependent decline of functional capacity and stress resistance, associated with increased risk of morbidity and mortality. In the present study, we screened several Natural Products (NPs) for a possible *in vivo* anti-ageing effect in *Drosophila* flies. Specifically, flies were cultured in the continuous presence of various doses of the NPs and the downstream effects on their physiology and lifespan were recorded. We identified a NP that prolonged both healthspan and lifespan of flies. Moreover, exposure of flies to this NP resulted in increased flies' muscle strength, higher activities of the main proteolytic systems, reduced proteome damage and increased anti-oxidant responses. These effects were mediated (at least in part) by activation of the Nrf2/Keap1 signalling pathway (a master regulator of cellular antioxidant responses) as they were abolished by RNAi-mediated Nrf2 knockdown in transgenic flies. Our on-going studies aim to identify the molecular target of this NP.

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P13 Characterization of lycopene biosynthesis and evaluation of antioxidant activity in fruits of five tomato varieties

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Lycopene is a naturally occurring chemical compound that gives fruits and vegetables a red/orange color. As an intermediate metabolite of β-carotene biosynthesis, lycopene represents one of the major carotenoids. Interest in lycopene is growing rapidly following the implication of lycopene in the prevention of cancer and cardiovascular diseases. The present study focuses on the molecular characterization of lycopene biosynthesis and antioxidant activity capacities of five commercially available tomato varieties (Cherry Ninolino F1, Elpida, Daphne, Eliseo Plum F1 and Oxheart) in three different tissues of tomato fruit: skin, flesh and seeds. Initially, gene expression profiles implicated in lycopene biosynthesis (*SlZDS* και *SlCRTISO*), and catabolism (*SlbLCY*) was investigated. For the molecular analysis, real-time PCR (qRT-PCR) was performed using *SlEF1a* as a reference gene. Differential regulation of the biosynthetic genes (*SlZDS* και *SlCRTISO*) was demonstrated in skin and flesh of all tomato varieties. Interestingly, the gene implicated in lycopene catabolism (*SlbLCY*) demonstrated a general induction in the tested tissues of the different varieties. Moreover, the antioxidant activity of the five tomato varieties was detected using two different antioxidant activity assays (FRAP and TEAC). Increased antioxidant activity was observed in the skin of the Cherry Ninolino F1 and Elpida varieties, whereas lower antioxidant activity was observed in the tested tissues of Daphne, Eliseo Plum F1 and Oxheart varieties. We are currently in the process of measuring the lycopene content in all samples under examination. The exploration of lycopene activity in different varieties and the 'selective' tomato consumption could be useful for the protection of human health.

P14

The macrophage-mediated regulation of fibrogenic response by murine fibroblasts is osteopontin-dependent

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Upon tissue injury, resident and infiltrating macrophages become activated into distinct subpopulations that sequentially orchestrate the restoration of tissue homeostasis. Soluble factors produced by macrophages may paracrinely affect adjacent fibroblasts, regulating tissue remodeling.

Osteopontin is expressed by activated macrophages and involved in macrophage function and gene expression. Experiments with osteopontin-deficient mice in inflammatory disease models mostly reveal a fibrosis-regulatory role for this molecule by as yet unidentified mechanisms. Accordingly, we recently identified osteopontin expressed by macrophages as an important promoter of cardiac fibrosis in a genetic heart failure model.

To unravel the complex pattern of macrophage regulation of fibrogenic response we stimulated macrophages obtained from wild type (wt) and osteopontin-deficient mice to assume pro-inflammatory (M1) or alternative (M2) activation status and exposed murine cardiac and lung fibroblasts to their conditioned media.

Compared to proinflammatory M1 macrophages, exposure to CM from M2 activated macrophages led to stronger fibrogenic response, assessed by collagen I and fibronectin 1 expression. Depending on cell culture density, M1 stimulation could lead to up-regulation of α-smooth muscle actin (α-SMA) expression, an indication of differentiation into myofibroblasts. Notaby, exposure to CM from osteopontin-deficient peritoneal macrophages resulted in lower levels of α-SMA by cardiac fibroblasts. Moreover, CM from wt macrophages induced fibroblast proliferation and in vitro wound closure, abilities impaired by osteopontin-deficient macrophages. A osteopontin-dependent macrophage regulation of fibrosis is proposed.

P15

A computational approach for integrating genome-wide chromatin profiles in the functional characterization of hematopoiesis

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Recent developments in next generation sequencing (NGS) provide the means for an accurate characterization of genome wide chromatin states, such as histone modifications, transcription factor (TF) occupancy profiles and gene expression levels. Application of NGS technologies in different cell types and/or differentiation states provide a highly accurate and highly comparable set of genomic maps that can be used to identify and characterize transcriptional regulatory events that underlie differential gene expression patterns in these cells. In this study we used a mathematical modeling and clustering approach to identify chromatin states that distinguish gene activity in the erythroid and megakaryocytic lineages during mouse hematopoiesis. More specifically, we used publicly available NGS datasets mapping a series of epigenetic modifications, TF occupancies and gene expression profiling in hematopoietic stem cells, in erythroblasts and in megakaryocytes. Differences in the epigenetic profiles of regions proximal to the transcriptional start sites (TSS) of genes were analyzed and related to differences in gene expression patterns using random forest regression models. Interpretation of the results provides insights into the role of the GATA-1 transcription factor in the erythroid versus megakaryocytic lineage specification. In addition, a role was revealed for the erythroid-related TFs Ldb1 and Scl/Tal1 acting further upstream the hematopoietic differentiation tree, prior to GATA-1 transcription activation at the common myeloid progenitor's stage.

P16

Divergent transcriptomic alterations induced by the Forced Swim Test in the hippocampus of high versus low novelty-seeker rats

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Susceptibility to stress and depression differs between individuals. The best animal model to study the neurobiology of affect regards spontaneous reactions to novelty. Rodents can be classified as high or low responders (HR, LR) based on the exploratory activity to stress of a novel environment. Our hypothesis is that phenotype-dependent changes in the gene expression of hippocampus could possibly accompany a distinct behavioural pattern in the forced swim test (FST). At 24 h post-FST, HR and LR rats (stressed and unstressed controls) were sacrificed and total RNA was extracted from hippocampal samples. Using whole rat genome Illumina arrays (RatRef-12 Expression BeadChip) we studied ~22.260 transcripts. Functional analysis into pathways and networks was performed using the Ingenuity Pathway Analysis and GeneCodis software. In the test FST session, HR and LR rats show similar "depressive-like" status but distinct behavioral pattern in the pre-test session. Interestingly, over twice as many genes were significantly changed following FST in LR, as compared to HR rats. Neurogenesis (*Ephb2,Nog,Ntf3 Tgfb1,Smad7,Sox2,Srr*) and synaptic plasticity-related genes (*Ephb2*, *Epha5, Ddn*, *Stx4*, *Pfn, Syt12*) were induced in the hippocampus of LR rats in response to FST, whereas in HR rats, several genes associated with the induction of apoptotic mechanisms (*Eif2ak3,Sgpp2,Mbtps2,Perk,Gsk3b,Mbtps2,Atf-6,Sgpp2,Rip1*) were found to be up-regulated. Based on our findings, we conclude that the hippocampal response to the same stress regimen is different between HR and LR rats at the transcriptional level, despite their seemingly similar "depressive-like" phenotype in the FST test.

P17 Dynamic miRNA profiling of BMP4 induced osteogenesis in bone marrow mesenchymal stem cells

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Mesenchymal stem cells (MSCs) are multipotent adult stem cells that can give rise to different cell types. Their differentiation fate depends on the signals received by their microenvironment and is regulated by growth factors modulating multiple pathways. Among factors involved in MSCs differentiation, Bone Morphogenetic Proteins (BMPs) have been shown to be involved in the commitment of MSCs towards the osteogenic lineage. In this study we sought to investigate alterations in the expression profile of miRNA during the BMP4 induced osteogenesis. In particular, bone marrow MSCs were cultured for 7, 14 and 21 days in the presence of BMP4. MSCs derived from six healthy individuals were used in our analysis. Samples were labeled and hybridized to miRXplore platform which is based on two channel microarray technology. The raw signal intensities for each time point were obtained with ImaGene software and data were pre-processed, normalized and analyzed with Gene ARMADA tool. Statistical analysis revealed a small number of miRNAs with differential expression among time points. The majority of differentially expressed miRNAs were upregulated upon BMP4 treatment. Potential targets of the significantly altered miRNAs were identified with DIANA software and further analyzed by StRAnGER algorithm, which performs Gene Ontology (GO)-based analysis to highlight the broader putative cellular regulatory network.

P18 Examining genetic ancestry and demographic history among HapMap phase III populations

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The genetic history of human populations is a complex mosaic affected by a number of factors such as migration events, population movements, population bottlenecks, expansions and other demographic events. The genomic footprint of these phenomena enables DNA-based investigation of historical events that involve population size, population composition as well as other significant components and provide us the opportunity for a more robust understanding and interpretation of historical events. Genomewide genotyping and sequencing datasets that are available today have the potential to contribute to a much greater understanding of critical aspects of human history. Here, we used methods that detect genetic ancestry, including principal component analysis, calculation of Fst values, phylogenetic tree representation and evaluation and detection and analysis of IBD (identity by descent) blocks in order to study genetic relationships of worldwide populations from HapMap III. We present genome-wide analysis on available data from 329381 Single Nucleotide Polymorphisms (SNPs) genotyped for 700 individuals (11 populations and 4 continental regions from HapMap III). We study the genetic structure of the HapMap III populations, providing valuable insight into the demographic history of populations worldwide.

P19

Inference of protein dynamics from Fluorescence Recovery After Photobleaching (FRAP) data reveals modified protein kinetics following whole-cell UV irradiation

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Fluorescence Recovery after Photobleaching (FRAP) is a functional imaging technique that permits the exploration of protein dynamics in living cells by allowing the estimation of kinetic parameters. Here, we use an *in silico* approach for the inference of kinetic parameters (namely diffusion coefficient, fraction of immobilization and residence times) from short and long FRAP experiments. Our inference method is based on a stochastic hybrid model of protein diffusion and binding in a realistic setup at a particle level and involves the construction of a mapping from FRAP recovery curves to the underlying protein kinetics using an Artificial Neural Network.

To validate the method, we analysed the kinetic behavior of the DNA replication and repair factor PCNA and the licensing factor Cdt1, both tagged with GFP, and GFPNLS as a control of a freely diffusing nuclear protein. All proteins were transiently expressed in unsynchronized MCF7 cells. Using our method, we extracted kinetic parameters before and after UV irradiation. More specifically, using short FRAP experiments (time frame of approximately 20 sec) we reconstructed purely diffusive behavior for GFPnls, transient interactions coupled to diffusion for Cdt1 and permanent interactions coupled to diffusion for PCNA following UV irradiation. To further challenge our method, for the case of PCNA following UV irradiation, we performed long-FRAP experiments (time frame of approximately 1000 sec) that allow the assessment of slower dynamics, unable to be experimentally observed within short time frames. The extracted kinetic parameters are largely reconfirmed, proving the robustness of our method regardless of the experimental setup.

P20

Catalytic properties of ribosomes from heterotic yeast strains exhibiting translational infidelity

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This study explores whether heterosis (hybrid vigor) affects protein synthesis. Heterosis is the increase in growth rate and fertility of F1 hybrids compared to their homozygous parents. Hybrids exhibit heterosis possibly because of specific heterozygosity of the controlling genomic regions. A genomic scan of homozygous and heterozygous yeast strains indicated several ribosomal protein genes in which allelic heterogeneity in hybrids is associated with increased growth rate. One such locus encodes yeast ribosomal protein L39. L39 affects the two main ribosomal activities, translational fidelity in a major way and peptide bond formation in a minor way. We showed recently that the hybrid strain has an increased error frequency (EF) of translation compared to either homozygous parental strain. However, the hemizygous strain carrying a deletion of one of the two L39 alleles possessed an even higher EF than the hybrid. This exceeds the expected dosage effect caused by deletion of one of the two L39 alleles. First, we show that cycloheximide, an inhibitor of protein synthesis, inhibits equally growth of parental, hybrid and hemizygous strains as well as growth of isogenic wild type strains. Next, we examined the possibility of a co-operative effect of heterosis and ribosomal protein L39 on the rate of peptide bond formation with the puromycin reaction. The k_{obs} of the hybrid strain at 0.4 and 2 mM puromycin are similar to those of the parental strains, whereas the k_{obs} of the hemizygous strain is slightly higher at both puromycin concentrations. These results allow investigation of a co-operative effect of heterosis and ribosomal protein L39

P21

Linezolid-dependent structure and function adaptation of ribosomes from nosocomial strains of S. epidermidis.

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We have recently reported (Pournaras et al., *Emerg. Infect. Dis.* 2013, 19:129-132) that the *Staphylococcus epidermidis* nosocomial strain (A2864) grows remarkably faster than the wild type strain in excess of the antibiotic linezolid, although it carries mutations in 23S rRNA associated to linezolid resistance. Linezolid is a synthetic oxazolidinone which targets the 23S rRNA and blocks petpidyltransferase activity. Although it was introduced very recently for the treatment of infections caused by Gram-positive pathogens, resistance attributed to specific mutations located mainly on 23S rRNA and L3 and L4 ribosomal proteins have been well documented. To investigate this paradox in molecular level, we isolated functionally active ribosomes from a wild type strain and we compared them with isolated ribosomes from the mutant strain grown in the absence and/or presence of linezolid. Interestingly, we observed essential differences in the structure and function of ribosome populations derived from the mutant strain which were assembled in the presence of linezolid. The catalytic activity of peptidyltransferase activity which is responsible for the peptide bond formation during translation and is expressed with the ratio k_{cat}/K_M was found significantly higher in the mutant strain in the presence of linezolid, but only for ribosomes assembled in the presence of the antibiotic. Moreover, the same ribosomes were unable to efficiently dissociate into subunits in the absence of the antibiotic. These observations imply that the presence of linezolid has modified the defined hierarchy of ribosomes assembly, leading to new functional species, active only in the presence of the antibiotic.

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P22

On the use of the antibiotic chloramphenicol to target polypeptide chain mimics to the ribosomal exit tunnel

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The ribosomal exit tunnel had recently become the centre of many functional and structural studies. Accumulated evidence indicates that the tunnel is not simply a passive conduit for the nascent chain, but a rather functionally important compartment where nascent peptide sequences can interact with the ribosome to signal translation to slow down or even stop. To explore further this interaction, we have synthesized short peptides attached to the amino group of a chloramphenicol (CAM) base, such that when bound to the ribosome these compounds mimic a nascent peptidyl-tRNA chain bound to the A-site of the peptidyltransferase center (PTC). Here we show that these CAM-peptides interact with the PTC of the ribosome while their effectiveness can be modulated by the sequence of the peptide, suggesting a direct interaction of the peptide with the ribosomal tunnel. Indeed, chemical footprinting in the presence of CAM-P2, one of the tested CAM-peptides, reveals protection of 23S rRNA nucleotides located deep within the tunnel, indicating a potential interaction with specific components of the ribosomal tunnel. Collectively, our findings suggest that the CAM-based peptide derivatives will be useful tools for targeting polypeptide chain mimics to the ribosomal tunnel, allowing their conformation and interaction with the ribosomal tunnel to be explored using further biochemical and structural methods.

P23

Post-transcriptional regulation of Alpha-synuclein expression

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Clinical, genetic and experimental evidences have linked alpha-synuclein (asyn) expression to Lewy body diseases, including Parkinson's disease and dementia with Lewy bodies. The aberrant function of asyn remains obscure but it is likely to stem from the excess accumulation of asyn species that form toxic aggregates in presynaptic terminals affecting neurotransmitter release. Several physiological and pathophysiological conditions that lead to lower levels of cap-dependent translation in cells such as differentiation, oxidative stress, hypoxia or nutrient limitation, often drive an alternative mode of translation which is dependent on Internal Ribosome Entry Site (IRES) sequences in the 5'UTR of specific mRNAs. IRESs form hairpin structures that attract eukaryotic ribosomal translation initiation complexes promoting translation initiation independent of the presence of the commonly utilized 5'-m7G cap structure. Here, sequence analysis of asyn mRNA revealed the presence of such an IRES sequence in the proximal 5'UTR. Experimentally, we provide evidence that asyn can be translated in cap-independent manner and we propose that this is maybe a mechanism that contributes to excess accumulation of asyn in pathological conditions.

P24 Secondary structure of the tRNA-dependent glyS T-box riboswitch

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T-Box riboswitches are important regulatory elements in the 5'UTR of mRNAs, which utilize charged or uncharged tRNA molecules as ligands. They are widely represented in Gram-positive bacteria and control the expression levels of enzymes involved in amino acid biosynthesis and aminoacyl-tRNA synthesis. In *Staphylococcus aureus*, a T-Box riboswitch precedes the *glyS* gene encoding glycyl-tRNA synthetase (GlyRS). GlyRS mediates the formation of the Gly-tRNA^{Gly} molecules that serve as substrates for the protein synthesis and for the exo-ribosomal glycine-mediated stabilization of the bacterial cell wall. Previous work of our group revealed that in *S. aureus* there are two encoded proteinogenic tRNA^{Gly} isoacceptors (P1 and P2) and three non proteinogenic tRNA^{Gly} isoacceptors (NP1, NP2 and NEW) with extra-translational roles which bind poorly to EF-Tu. Recently, we cloned the *S. aureus glyS* T-box. Detailed RNA structural probing and primer extension analysis unravelled the structural folding of the *glyS* T-box alone or in the presence of the P1 tRNA$^{Gly(GCC)}$, in order to verify that the stem I loop in the *glyS* T-Box adopts the tRNA^{Gly} structure. Here we propose the biochemically defined secondary structure of this specific regulatory tRNA-dependent element. Our goal is the biochemical and structural characterization of the interaction of all above mentioned tRNA^{Gly} isoacceptors, with the *glyS*-specific T-box from *S. aureus*, both *in vitro* and *in vivo*. The *glyS* T-box is a characteristic example of regulation at the transcription level and moreover represents a novel molecular target for antibacterial compounds since in this organism regulates to distinct however metabolically related tRNA-dependent networks.

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P25 Two spermidine analogues of chloramphenicol with diverse mode of action against ribosomal peptidyltransferase activity

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Chloramphenicol (CAM) is a broad spectrum antibiotic. It resembles the 3′-end of the aminoacyl-tRNAs and inhibits the catalytic activity of the prokaryotic ribosomes. It is known that polyamines favor the binding of CAM to ribosomes. This prompted us to synthesize two analogues of CAM, harboring one spermidine unit conjugated through its N^1- (F5) or N^4-amino group (KA-240) to the dichloro-methyl edge of the CAM-scaffold. Previous kinetic studies have revealed that both analogues, compared to CAM, exhibit superior activity, behaving as competitive inhibitors of the peptidyltransferase activity, following a two-step, slow-binding mechanism. To identify binding sites of F5 and KA-240 in the ribosome, we further investigated their binding process by time-resolved footprinting analysis. The results confirmed that both CAM-analogues bind initially to a hydrophobic crevice composed of nucleotides located adjacently to the A-site (A2451, G2505, U2506), thus explaining the competitive character of action. Soon after, they shift to a final position of high affinity, in which the nitrobenzene ring of analogues continue to interact with A2451, a nucleotide located at the heart of the A-site. Nevertheless, while the polyamine tail of KA240 orientates toward nucleotide A2062 through which allosterically affect the entrance of the ribosomal exit tunnel, the polyamine tail of F5 interacts with nucleotides A2062 and U2585 implicated in the rotary motion of the A-site substrate. Conclusively, although both CAM-analogues behave as competitive inhibitors of peptidyltranferase, their exact mode of action differs.

P26

Expression of miR-224, a novel molecular tissue biomarker predicting short-term relapse and poor overall survival in colorectal adenocarcinoma patients, is negatively correlated with mRNA levels of kallikrein-related peptidases 6 and 10

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Introduction: Colorectal adenocarcinoma constitutes the most frequent form of colorectal cancer. We analyzed miR-224 levels in colorectal cancer adenocarcinoma samples and paired non-cancerous mucosa, in order to examine the prognostic value of miR-224 as a novel molecular tissue biomarker in colorectal adenocarcinoma. Moreover, we analyzed the mRNA expression of four kallikrein-related peptidases (*KLK*s), including *KLK4*, *KLK6*, *KLK10*, and *KLK11*, to investigate whether they could constitute potential targets of miR-224.
Materials and Methods: Total RNA was extracted from 119 primary tumors and 70 paired non-cancerous mucosa of CRC patients. 2μg of total RNA were polyadenylated using poly(A)-polymerase and then cDNA was prepared by reverse transcription using an oligo-dT-adaptor primer. A highly sensitive quantitative real-time PCR methodology (qRT-PCR), based on SYBR Green chemistry, was developed for miR-224. Moreover, qRT-PCR protocols - previously developed in our lab – were used for mRNA expression analysis of *KLK*s.
Results: miR-224 levels were significantly higher in colorectal adenocarcinomas than in non-cancerous counterparts (P<0.001). Elevated miR-224 expression predicts short-term relapse and poor overall survival in these patients (P=0.008 and 0.002, respectively), independently from established clinicopathological parameters. Furthermore, negative correlations were observed between miR-224 and *KLK6* mRNA (r_s=–0 .458, P<0.001) as well as *KLK10* mRNA levels (r_s=–0 .333, P<0.001).
Conclusion: miR-224 expression is negatively correlated with *KLK6* and *KLK10* mRNA expression and can be considered as a novel molecular biomarker predicting poor outcome in colorectal cancer patients.
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P27

miR-182 constitutes a novel molecular tissue biomarker predicting unfavorable outcome in colorectal cancer patients

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Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed malignancy worldwide and the fourth most common cause of cancer death. MicroRNA-182 (miR-182) is an oncogenic miRNA promoting metastasis. We analyzed the levels of miR-182 in CRC samples and paired non-cancerous mucosa, in order to examine the prognostic value of miR-182 as a novel molecular tissue biomarker in CRC.
Materials and Methods: We developed a highly sensitive quantitative real-time PCR methodology (qRT-PCR), based on SYBR Green chemistry. After the determination of the detection limit and the validation of our newly developed model, we extracted total RNA from 116 primary tumors and 60 paired non-cancerous mucosa of CRC patients. 2μg of total RNA were polyadenylated using *E. coli* poly(A)-polymerase and then cDNA was prepared by reverse transcription using an oligo-dT-adaptor primer. Finally, we applied the aforementioned method for the expression analysis of miR-182 in CRC specimens and undertook extensive biostatistical analysis to evaluate our results.
Results: miR-182 expression was upregulated in colorectal tumors, compared to non-cancerous paired mucosa (P<0.001), and also differed significantly among CRC patients with well-, moderately- and poorly-differentiated colorectal tumors (P=0.013). miR-182 overexpression is associated with high tumor extent (P=0.037), positive nodal status (P=0.006), and advanced TNM stage (P=0.003), and predicts independently poor overall survival in CRC (HR=2.53, P=0.048).
Conclusion: High miR-182 expression can be considered as a novel, unfavorable molecular tissue biomarker predicting poor outcome in CRC.

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P28

Symmetric division and post-mitotic asymmetries in embryonic stem cells

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Pluripotent cells are thought to enter the differentiation process by dividing asymmetrically: one of the daughter cells produced by an asymmetric mitosis maintains its full developmental potential, while the other cell exits the self-renewing state and proceeds to lineage commitment. Asymmetric cell division has been documented experimentally in adult stem cells and multipotent progenitors, but it is not clear whether it occurs in naïve embryonic stem cells. To address this question, we have studied the division of mouse stem cells (E14 cells) by live cell imaging and examined the segregation of specific cellular proteins in pairs of daughter cells. The results show that E14 cells divide symmetrically in the presence or absence of LIF. Symmetric division entails the equal distribution of pluripotency factors (Nanog, Sox2), fate and polarity determinants (Numb, aPKCζ) and lineage-specific markers (Brachyury) after completion of mitosis. Mitotic E14 cells feature a symmetric spindle and show no apparent asymmetries at the level of the nuclear envelope. However, longer monitoring after completion of mitosis reveals that primary cilia developed in daughter cells recruit Sonic Hedghog mediators (Gli2) in a non-synchronous fashion. This "asymmetry" develops after induction of in vitro differentiation and is never observed when the cells are maintained in their "ground state". From these data we conclude that a functional asymmetry may arise post-mitotically on the grounds of the non-synchronous assembly and/or activation of primary cilia during the G1 phase.

P29

"Intelligent" Materials supporting the differentiation of mesenchymal and other adult cells to osteoblasts

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Reversine (2-(4-morpholinoanilino)-N6-cyclohexyladenine) is a small purine-analogue synthetic molecule that is capable of dedifferentiation of lineage-committed cells of mesenchymal origin to a more primitive pluripotent state and BMP2 is a **B**one-**M**orphogenetic **P**rotein that is strongly involved in mesenchymal stem cells differentiation to osteoblasts. The purpose of this study is to investigate reversine's and BMP2's capability to cause differentiation of mesenchymal cells to osteoblasts, while being attached and functional immobilized to specific treated surfaces with immobilized tetramer streptavidin. Reversine and BMP2 protein were biotinylated *in vitro* and *in vivo*, respectively in order to attach to surfaces due the highly strong non-covalent bond of streptavidin-biotin. Our current experiments exhibit interesting results since the progress of differentiation "demands" BMP2 or reversine separately. On the contrary their potential towards differentiation is negatively influenced when both are added to the cell culture. In details,dental mesenchymal cells and adipose stem cells were treated with 100 nM reversine or 50 ng/mL BMP2 and then cultured under osteoblast-inducing conditions for 7 days. Reversine was also used to dedifferentiate lung cells (MRC5 line), which originate from the endoderm during embryogenesis, to osteoblasts which are of mesodermal origin (previous studies were made only for dedifferentiation of mesodermal cells). Alkaline phosphatase (AP) and Alizarin Red staining were used to detect the formation of osteoblasts, since they are specific markers of the osteoblast cell line. The results showed positive staining for AP and Alizarin Red, which indicate the successful osteoblast formation and they were also confirmed with RT-PCR for the osteoblast marker osteocalcin (OC). At the moment the differentiation processes are being studied direct on the biofunctionalized surfaces of "Intelligent materials".

P30 Corticotropin-Releasing Hormone (CRH) deficiency decreases neurogenic activity in adult hippocampus causing memory deficits

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Stress exerts differential effects on adult neurogenesis primarily via its stimulation of glucocorticoid release. Despite the negative effects of stress on progenitor cell proliferation in the hippocampus, challenges leading to robust increase in glucocorticoid levels have been reported to promote neuronal growth. We have previously shown that Corticotropin-Releasing Hormone (CRH), a main hormonal mediator of stress response, may overcome the negative effects of glucocorticoid and induce proliferation of neural progenitor cells in the embryonic brain. In order to elucidate the role of CRH in adult neurogenesis, we examined the proliferating activity of neural stem cells in the adult hippocampus of CRH-deficient (*Crh-/-)* and wild type mice. Our results showed that the number of BrdU- positive cells in the dentate gyrus of the *Crh-/-* mice were significantly reduced compared to the wild type group. Since hippocampal neurogenesis is linked to memory, we subjected *Crh-/-* and wild type mice to novel object recognition and novel object location tasks that assess short-term memory. Although *Crh-/-* mice possessed normal object recognition memory, their object location memory was impaired, indicating a hippocampal-dependent cognitive deficit. CRH receptors were detected in adult hippocampal neural stem cells in support of the possibility of direct effects of CRH on this neurogenic population. These findings suggest new physiological roles for CRH in adult brain adaptive responses to stress stimuli

P31 Endothelium positively regulates cardiac progenitor cells in differentiating ESCs.

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Formation of the cardiovascular system is one of the earliest differentiation events during embryogenesis. During heart development endothelial-cardiomyocyte interaction is essential for the survival, growth and contractile activity of cardiomyocytes. However, little is known about the specific nature of the functional interaction between endothelium and cardiomyocytes during early cardiomyocyte lineage specification. We use an *in vitro* model system of mammalian embryogenesis, the differentiation of embryonic stem (ES) cells through embryoid bodies (EBs) formation in the presence of growth factors that favour mesodermal differentiation. Under these culture conditions cardiac differentiation was very efficient reflected in the high potential of EBs to give rise to beating cardiomyocytes (88%).We went on to test whether cardiomyocyte differentiation was affected under conditions that perturbed vascular formation and organization in EBs. We achieved this by expressing a mutant of Vascular Endothelial Cadherin, ΔNEXD-VEC, under the control of the promoter of VE-Cadherin (Pvec) in a tissue specific manner. Expression of this dominant negative mutant is known to disrupt adherens junctions in general. We demonstrate that expression of ΔNEXD-VEC under the control of Pvec causes not only impairment of endothelial cell assembly and lack of vascular structure formation in EBs, but also has a profound effect on cardiomyocyte differentiation. Mutant EBs show a striking loss of beating phenotype and this effect appears to be the result of a substantial deficiency in the formation of cardiac progenitors.

P32

Functionalized gold nanoparticles for treatment of central nervous system injuries

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The inability of the mammalian central nervous system (CNS) to regenerate after injury causes functional impairment. During the acute phase after spinal cord injury (SCI), disruption of cell membranes leads to excitotoxicity, oxidative stress, Ca^{2+} influx, inflammation and cell death, resulting to the detrimental secondary neurodegenerative events of the chronic phase. Nanotechnology offers the possibility of a superior drug delivery platform that may be adapted to the clinic. In particular, colloidal gold nanoparticles (AuNPs) appear as leading candidates in the field of nanomedicine due to their inert and non-immunogenic properties, good biocompatibility, ease of preparation and modification towards several functionalities. Previous studies have shown that poly-ethylen-glycol (PEG) administration in CNS injury models can restore action potentials and induce functional recovery due to its membrane sealing potential. Yet there are serious limitations, arising mostly from PEG toxicity and limited bioavailability when administered systemically. To overcome this drawback we developed PEG (MW 2000)-functionalized 40-nm-diameter AuNPs for in vivo use as a key treatment to seal disrupted cell membranes at early stages after SCI. Our results show that PEG-functionalized AuNPs delivered in the lesioned spinal cord caused no adverse effects, such as body-weight loss or animal death when compared to control vehicle-injected mice. Behavioral analysis of locomotor function revealed significantly greater recovery in the PEG-AuNP treated group as compared to controls, up to 8 weeks post injury. Additionally, immunohistological analysis indicated attenuated inflammatory response and enhanced motoneuron survival. Our data show prospects of an efficient drug carrier platform applicable to the clinic.

P33

Investigating the role of desmin in the characteristics of adult cardiac Side Population stem cells

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Desmin, a muscle-specific Intermediate Filament protein, apart from its mitoprotective and cardioprotective role, it has been proposed to have a crucial role during commitment and differentiation of cardiomyocytes. Recently, it has been identified in the cardiac Side Population of stem cells of the adult mouse heart (SP cells). Considering the above, we hypothesize that the absence of desmin may also affect the characteristics of the SP cells. To address that, we compared the phenotypic and genotypic characteristics of wt and desmin null (des-/-) adult cardiac SP cells. During the phenotypic characterization, we determinedthe expression of various cell surface markers (Sca1, Thy, CD31, CD34)of different cell types that exist within the heart between wt and des-/- SP cells, in order to identify the endogenous cardiac Side Population ($Sca1^+/Thy1^-/CD31^-/CD34^-$ SP) and the effect that the absence of desmin may have on these subpopulations. Our data showed that the luck of desmin affects the SP population as the number of SP and $Sca1^+$ SP cells was significant elevated, whereas the number of $Sca1^+/Thy1^+$, $Sca1^-/Thy1^+$, $Sca1^-/CD31^+$ and $Sca1^-/CD34^+$ SP cells was significantly lower in des-/- mice compared to wt. During the genotypic characterization we addressed the expression profile of gene markers of stem cell and differentiated cardiac cells in order to determine the commitment level of SP cells. The results showed asignificant downregulation of Nkx2.5 cardiac specific transcription factor in des-/- SP cells after culturing for 7 days, indicating a potential role of desmin during SP commitment.Funded by Synergasia 09SYN-12-966 grant.

P34

New generation S/MAR based episomal vectors, with high potential for gene transfer into hematopoietic progenitor cells

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The episomal vector pEPI-eGFP contains eGFP as reporter gene and Scaffold /Matrix Attachment Region (S/MAR), an element facilitating episome retention in the host cell nucleus. This vector is not capable for stable transfection of hematopoietic progenitor cells CD34+. We introduce two modifications: Firstly, we substituted promoter CMV of eGFP with SFFV (pEPI-SFFV) or the hybrid promoter EF1/HTLV (pEPI-EF1/HTLV) that function more efficiently in CD34+ cells. Secondly, the replication initiation region (IR) from the β-globin gene locus was added in the SFFV containing plasmid (pEPI-IR), to enhance the episome's replication capacity. The optimal insertion site for IR was determined by Stress Induced Duplex Destabilization (SIDD) analysis. Plasmids were successfully transferred into K562 cells as determined by flow cytometry. All cell lines retained the episome for five months continuous culture, with and without selection with G418. Subsequently, plasmids pEPI-SFFV, pEPI-IR and control plasmid pEPI-eGFP were used to transfect CD34+ cells from adult peripheral blood, from mobilised healthy donors. Replacement of CMV promoter by either EF1/HTLV or SFFV promoter has a positive, measurable effect on plasmid establishment Inclusion of the IR element brings about a further, significant increase-more than double- in the establishment rate of the vector. Furthermore, vector pEPI-EF1/HTLV and vector pEPI-SFFV have almost the same capacity for supporting eGFP expression per plasmid copy, while vector pEP-IR is capable for 3 times higher eGFP expression per plasmid copy, than cells carrying either of the other two vectors. The new vectors show great promise for episomal gene transfer into human haematopoietic progenitor cells.

P35

Proteomic evaluation of decellularisation protocols in umbilical cord artery

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Introduction: Major achievements in creating decellularized whole tissue scaffolds have drawn considerable attention to decellularization as a promising approach for tissue engineering. In this study histological and proteomic analysis was performed to evaluate the efficiency of the two decellularization protocol.
Methods and Results: In decellularization protocol A, umbilical arteries (n= 20) were incubated in CHAPS and sodium dodecyl sulfate followed by incubation in α-MEM with foetal bovine serum. In decellularization protocol B the umbilical arteries (n=20), were incubated in Hypotonic Tris and SDS followed by incubation in Nuclease solution. Decellularized umbilical arteries were completely devoid of cellular and nuclear material while extracellular matrix remained intact Native and decellularized arteries were digested using Proteinase K and DNA content was measured. For the proteomic analysis the umbilical arteries were snap frozen in liquid nitrogen and homogenized. The protein content was measured using Bradford assay and analyzed by 2D Electrophoresis. Protein spots were excised manually and tryptic digestion and Peptide Mass Fingerprinting was performed. Decellularized patches (3x3 mm) of umbilical artery were seeded with 10,000 Wharton Jelly-MSCs-RFP$^+$ per patch, incubated for 2 weeks at 37^o C and 5% CO_2. and no cytotoxic effect was observed.
Conclusion: Both decellularization protocols effectively removed the cellular material while the ECM remained intact and supported MSCs seeding.Future studies are warranted to elucidate the specific effects of altered structure–function relationships on the overall fate of decellularized umbilical arteries.

P36

The role of PML in leukemia and embryonic stem cells

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Embryonic (ES) and adult stem cells share many properties with cancer stem cells, cells capable of initiating and sustaining a tumor that are rendering many forms of cancer resistant to chemotherapy. Unique stem cell features include quiescence (the slow cycling of a stem cell), self-renewal potential and pluripotency, e.g. the ability to generate a phenotypically heterogenous progeny. Recently, tumor suppressor proteins like PTEN and PML were recognized as important quiescence factors in hematopoietic stem and leukemia initiating cells. The Pml gene was first identified in a chromosomal translocation involved in acute promyelotic leukemia. It encodes the core component of the so-called PML-nuclear bodies affecting a number of cellular processes including apoptosis, cellular proliferation, senescence and stemness. Its depletion disrupts normal mammary gland development, affecting differentiation of cell subpopulations within the developing gland. It also modulates cell fate during neocortex development by regulating cell cycle progression in concert with pRB. Previous reports using p19 embryonal carcinoma and ES cells showed that Nanog and Oct4 – key regulators of pluripotency- associate with PML protein which is required for the expression of Oct4. In an effort to understand the molecular mechanisms underlying the involvement of PML in quiescence, proliferation and differentiation of embryonic and cancer stem cells we studied the effect of PML gain/ loss in mouse ES cells and in leukemic monocytes.

P37

Cell cycle control and checkpoint responses following UV-irradiation in mouse embryonic stem cells

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Mouse Embryonic Stem Cells (MESCs) have the ability to proliferate and self-renew indefinitely in culture and, when stimulated, to differentiate towards all three germ layers. The cell cycle of MESCs differs from the cell cycle of committed cells: it is very rapid while major checkpoint responses have been reported to be absent. Maintaining genome stability is pivotal for embryonic stem cells, as they give rise to all mature cell types. We are interested to understand the molecular mechanisms which ensure genome stability in MESCs, and how these compare to controls acting in differentiated and cancer cells.

We show that MESCs have a very short G1 phase and move to S-phase with high synchrony following mitotic arrest. During S-phase, replication factories are visualized by immunofluorescence against the replication protein PCNA. They show characteristic early, middle and late S-phase localization, reminiscent of replication factory dynamics in differentiated and cancer cells. Following UV-irradiation in G1 phase, MESCs arrest and do not progress into the following S-phase. This shows that a DNA damage response recognizing UV-damaged DNA and blocking cell cycle progression is operative in MESCs. We show that the DNA replication licensing factor Cdt1 is specifically expressed during the G1 phase in MESCs. Following UV-irradiation during G1, Cdt1 is rapidly proteolysed. Cdt1 is however protected from proteolysis when MESCs are irradiated in mitosis. Our data show that at least some checkpoint responses are operative in MESCs and suggest that Cdt1 proteolysis following DNA damage in G1 may contribute to the maintenance of genome stability in MESCs.

P38

Order and disorder in a nuclear envelope protein: A structural and computational study of Lamin B Receptor

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Lamin B Receptor (LBR), a ubiquitous integral protein of the nuclear envelope is thought to participate in a variety of nuclear functions, including tethering of the nuclear lamina to the inner nuclear membrane and "transient trapping" of nuclear components that are involved in chromatin remodeling and transcriptional inactivation. The amino-terminal part of the protein, which has been shown to mediate most of LBR interactions, harbors a 40-residue region rich in Arg-Ser repeats that has the typical features of an IDP protein and is flanked between two globular domains (a Tudor domain and a 110-amino acid segment with no apparent homologues). The RS-rich region is highly charged and punctuated by multiple phosphorylation sites. We have shown that this region is responsible for multiple LBR interactions, including its homopolymerization. However, its conformational and functional properties are most likely modulated by the type and the extent of post-translational modifications, mainly phosphorylation and methylation. Here, we present the results of a biochemical, biophysical and computational study of LBR regions addressing the effect of physiologically relevant PTMs and inter-domain interactions on LBR structure and function.

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P39

A comparative nmr study of four viral macro domains

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Macro domains are ancient and widely distributed throughout all eukaryotic organisms, bacteria, and archaea, indicating a ubiquitous and important basic biological function. Macro domains are also found in nonstructural proteins (nsPs) of several positive-strand RNA viruses, including hepatitis E virus, rubella virus and coronaviruses, as well as alphaviruses. The functions of the macro domain are poorly understood, but it has been suggested to be an ADP-ribose-binding module.

In this study we apply NMR spectroscopy to study the conformational properties and dynamics of four macro domains: (a) two from New World alphavirus (Mayaro & Venezuelan equine encephalitis virus), (b) one Old world alphavirus (Chikungunya virus) and (c) one from the Hepevirus genus (HEV-1).

The four macro domains are cloned and expressed with Poly(His)tag, in high yields in *E. Coli.*. All the protein constructs are soluble and using *E.coli* culture supplements prior to induction in typical (M9) minimal media the bacteria growth rates and protein yield were generally increased. Initial 1D ^{1}H & 2D ^{1}H-^{15}N HSQC NMR experiments suggest that all four macro domains are folded in solution. Acquisition and analysis of 2D/3D homo/heteronuclear NMR data are underway.

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P40

BRCA1-BRCT cancer-related point mutations alter subcellular localization of BRCA1 in vitro

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The *brca1* is one of the most extensively studied genes related to breast and ovarian cancer. A large number of missense mutations are located at the C-terminal tandem repeats (BRCT domain) of the BRCA1 protein, a multifunctional domain involved in the interaction of BRCA1 with partner proteins. The M1775K and V1809F mutations, detected in breast tumor patients, were shown to either lower the stability of the BRCT domain and/or disrupt BRCT interaction with synthetic phosphopeptides.

Based on these data we sought to determine whether the M1775K and the V1809F BRCT destabilizing mutants do alter BRCA1 function, as monitored by BRCA1 movement into the nucleus upon DNA damage. The subcellular localization of BRCA1 wt and mutant proteins, fused to EGFP, was analyzed in MCF-7 cells, in the presence or absence of genotoxicity. EGFP-M1775K and V1809F BRCA1 mutants were unable to enter the nucleus, even after DNA damage, in contrast to either the EGFP-BRCA1wt or the less affected variant M1652I, while under the same conditions p53 and RAD51 moved to the cell nucleus characteristically. These results indicate that the functional changes due to the disruption of the C-terminal folding might be crucial for BRCA1 nuclear transport.

In conclusion, it seems that the impact of the integrity of the BRCA1-BRCT domain in structural level is crucial for proper function of the protein, as shown by the modifications in its subcellular localization and may contribute to the deficiency of DNA repair and cell cycle control processes observed in breast cancer cells.

P41

Conformational dynamics of N-/C- terminal domains of anthrax lethal factor metalloprotease

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In the pathogenesis of Anthrax disease, the bacterium's lethal toxin (LeTx) is of critical importance, while its component called Anthrax Lethal Factor (LF), 90 kDa Zn-dependent highly specific metalloprotease, is particularly interesting [Pannifer AD et al Nature 2001, 414, 229.]. Its proteolytic activity is targeted in a highly specific way towards vital cellular signal transducers, the family of mitogen-activated protein kinase kinases (MAPKKs).

In order to elucidate how LF participates in the formation of (LeTx), as well as its catalytic site structural-functional activity towards its kinase substrates, we attempt the expression and NMR study of Domain I (233 a.a.) and Domain IV (225 a.a.) polypeptides. Structural analysis of these domains will be helpful in an effort to inhibit both these processes, as part of a possible therapeutic approach. In this regard, possible peptides that may antagonize MAPKKs in binding to the catalytic centre, thus preventing their pathological proteolysis, are also designed and expressed. Different experimental protocols are currently applied for the effective overexpression and labelling of these polypeptides, before the application of multi nuclear and multi dimensional NMR spectroscopy. Preliminary results suggest that the recombinant 15N-labelled N-terminal polypeptide is soluble and folded in solution and suitable for NMR conformational analysis. On the contrary, the C-terminal polypeptide, while expressed in abundance, seems to be almost totally insoluble, thus making NMR study in solution impossible. Efforts are done to overcome this hurdle.

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P42

Drug solubility measurement; an ultimate tool for biological assays

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Tumor Necrosis Factor is a trimeric cytokine which has been associated with the inflammatory response to tissue injury and various viral - bacterial infections. It is found to possess a key role in Rheumatoid Arthritis (RA) pathogenesis. According to the traditional anti-TNF and anti- RANKL treatment of RA, our purpose is the extra cellular inhibition of this pro inflammatory cytokine as an effective therapy. The final goal is the discovery of molecules with inhibitor activity (lead compounds). These small molecules interact with protein trimers, promote trimer dissociation and therefore, an inactivated dimer arises. RA lead project aims at discovering and developing new inhibitors with a focus on *in silico* drug design and optimization of candidate hits, organic synthesis and *in vitro – in vivo* evaluation of potential ligands. A major issue that emerged during this study was the low aqueous solubility of newly synthesized compounds. It is not coincidental the fact that drug solubility is an area of drug discovery and development that attracts growing concern, while solubility affects directly bioavailability. Our efforts are targeted towards not only the development of solubility measurement methods (such as HPLC and direct UV) but also drug solubility enhancement using “protein friendly” co solvents. Further to solubility measurement, we also intend to create a solubility prediction model based on our results. The findings of this research will have great impact on the development of binding and biological assays as well as compound selection during discovery process.

P43

Expression optimization of a soluble, active recombinant human Cyclin A in Escherichia coli, refolding screening and characterization with putative inhibitors

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Cyclin-dependent kinases as well as their activation partners, Cyclins have been widely held as anti-cancer targets, due to their regulatory role in cell cycle. In this work we attempt the over-expression of recombinant truncated forms of human cyclin A, seeking the high levels of yield and purity required in our attempt to crystallize its complexes with their cell cycle regulatory partners. The truncated Cyclin A2 (amino acids 174-432)) have already been generated from cDNA and fused to the appropriate bacterial vector. The major objective during the course of this work is to obtain the protein in a soluble and stable form for crystallization and characterization purposes. Implementing various strategies for protein refolding, we performed a series of experiments to determine the optimum conditions. Following over-expression in *E. coli* cells, the recombinant proteins from inclusion bodies were refolded, towards higher yield and purity and were characterized with putative inhibitors in the form of existing synthesized peptides.

P44 Fragment-based design for new antidiabetic agents: revisiting the 3D structure of glycogen phosphorylase with known lead compounds

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Despite the increase on the approved drug treatments for type 2 diabetes, the side effects of these treatments remain a challenge. Glycogen phosphorylase is an enzyme directly implicated in glycogen metabolism and has served as a validated target for the development of new hypoglycaemic agents applying the structure-based drug design approach. A large number of ligands targeting its distinct binding sites have been investigated in complex with the enzyme with the aid of X-ray protein crystallography and the results have already guided the design and synthesis of compounds in the nM range [1]. With the aim to delineate the structural features that promote binding, a hybrid method of fragment-based screening and rational drug design has been applied utilizing the fundamental knowledge derived from previous studies [2]. Kinetic studies followed by high-resolution structural studies using synchrotron radiation at beamline P14, Petra III, EMBL-Hamburg Unit, of five fragments emerging from known inhibitors of glycogen phosphorylase provide an interesting paradigm in the quest for new lead molecules.

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P45 Functional dissection of the adenine-guanine-hypoxanthine transporters of Escherichia coli

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The evolutionarily ubiquitous family NCS2 (Nucleobase:Cation Symporter-2) encompasses transporters that are conserved in binding-site architecture but diverse in substrate selectivity. Putative purine transporters of this family fall into one of two homology clusters, COG2233, represented by well studied xanthine and/or uric acid permeases, and COG2252, consisting of transporters for adenine, guanine and/or hypoxanthine which remain unknown with respect to structure-function relationships. We analyzed the COG2252 genes of *Escherichia coli* K-12 with homology modeling, functional overexpression and mutagenesis and showed that they encode high-kinetic affinity permeases for the uptake of adenine (PurP, YicO) or guanine and hypoxanthine (YjcD, YgfQ). The two pairs of paralogs differ clearly in substrate and ligand preferences. Of 25 putative inhibitors tested, PurP and YicO recognize with high affinity N^6-benzoyladenine, 2,6-diaminopurine, and purine, YjcD and YgfQ recognize 1-methylguanine, 8-azaguanine, 6-thioguanine, and 6-mercaptopurine, and no analog is a high-affinity ligand for both transporter types. PurP and YjcD were subjected to site-directed mutagenesis at highly conserved sites of transmembrane segments of the core domain. Residues irreplaceable for uptake activity or crucial for substrate selectivity were found at positions occupied by similar-role amino acids in xanthine- or uric acid-transporting homologs and predicted to be at or around the binding site. Our data indicate that the distantly related transporters of COG2233 and COG2252 use distinct but topologically equivalent side chains to dictate the binding-site function and selectivity. Based on our functional studies, we propose renaming of genes *purP*, *yicO*, *yjcD* and *ygfQ* to *adeP*, *adeQ*, *ghxP* and *ghxQ*, respectively.

Acknowledgment: *This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: THALES, Investing in knowledge society through the European Social Fund.*

P46

NMR CONFORMATIONAL DYNAMICS OF ARKADIA & ARKADIA-2 E3 UBIQUITIN LIGASES ring domainS

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E3 ubiquitin ligases play a key role in the proteolytic degradation of proteins through the Ubiquitin-Proteasome pathway [Hershko A & Ciechanover A, Annu Rev Biochem 1998, 67, 425]. ARKADIA is the first example of an E3 ligase that positively regulates TGF–β family signaling through its C-terminal RING domain [Episkopou V et al. PLoS Biol 2007, 5, e67], while its homologue, ARKADIA-2, is implicated in BMP pathway.

The ARKADIA RINGs, were cloned and expressed in their Zn-loaded form and studied through NMR Spectroscopy [Kandias NG et al. *BBRC* **2009**, 378, 498]. The 3D NMR solution structure of ARKADIA-1 RING was determined and deposited in PDB (2KIZ). Additionally, NMR-driven titration studies were also performed to probe the interaction interface of ARKADIA-1 RING and the partner E2 (UbcH5B) enzyme and the RING-E2 complex was constructed through an NMR-driven docking [Chasapis CT et al. *Proteins* **2012**, 80, 1484].

Additionally, this study resulted to the identification of ARKADIAs RING functionally important residues, such as the conserved Trp972. Trp972 is considered as one of the key residues for E2 recognition and binding [Huang A, et al. J Mol Biol 2009, 385, 507]. According to recent experimental evidence, the mutation of the Trp972 to Arg abolishes the ability of ARKADIA to amplify TGF-b-Smad2/3 signaling responses in tissue culture transcription assays [Episkopou V, et al. Cancer Res. 2011, 71, 6438]. Various ARKADIA Trp mutants are now being studied through NMR in order to obtain an atomic-level insight about the structural base of ARKADIA-1 & -2 RING capability to selectively interact with the appropriate E2.

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P47

NMR INSIGHTS ON THE CONFORMATIONAL PLASTICITY OF THE EXTRACELLULAR DOMAIN OF A PROKARYOTIC nAChR HOMOLOGUE

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Pentameric ligand-gated ion channels (pLGICs) of the Cys-loop family are transmembrane glucoproteins, which are important regulators for the rapid chemo-electrical transduction; however, the ion permeation and gating mechanisms of these membrane proteins remain elusive. Recently, the X-ray structures of two prokaryotic homologues of the nicotinic acetylcholine receptor (nAChR), which is the most studied member of the LGIC family, have been determined. The first is the bacterial Gloeobacter violaceus pentameric LGIC homologue (GLIC) studied at 2.9 Å resolution in an apparently open conformationa and the second is the bacterium Erwinia chrysanthemi (ELIC) pentamer, studied at 3.3 Å resolution defining a closed conformation of the channelb. It is interesting to note that the extracellular soluble domain of GLIC has been found to remain in monomeric state in solution, though it assumes a hexameric quaternary structure when is crystallizedc.

The 200-residue extracellular domain of GLIC was cloned and expressed in high yields in *E. coli.* The ^{1}H-^{15}N HSQC exhibits signal dispersion typical for polypeptides with mainly beta structure. $^{13}C/^{15}N$ labeled GLIC was studied using heteronuclear multidimensional NMR spectroscopy and <40% of the backbone nuclei were originally identified. Deuterated, triple labeled ^{13}C, ^{15}N and ^{2}H samples were used for the acquisition of triple-resonance NMR spectra and 50-60% of the backbone resonances have been identified by the analysis so far. Selective ^{15}N labeling and unlabeling techniques were utilized for 12 different amino acids in prototrophic and auxotrophic *E.coli* strains aiming at the identification of approximately 80% of the backbone resonances.NMR data suggested that various GLIC segments are characterized by conformational exchange behavior. NMR data in higher temperatures, H/D exchange experiments and ^{15}N relaxation measurements were used to determine the dynamics of the protein and the determinants of the $GLIC_{ECD}$ assembly and oligomerization[d].

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P48

Friend of GATA-1 (FOG-1) interacts with the cohesin complex and CTCF in erythroid cells.

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The Friend of GATA-1 (FOG) family of zinc finger proteins play essential roles in development and cell differentiation through physical interaction with GATA transcription factors. In hematopoiesis, FOG-1, like its interacting partner GATA-1, is necessary for lineage specification towards the erythroid and megakaryocytic cell fate and is involved in both transcriptional activation and repression, potentially through DNA looping. Using a biotinylation tagging approach in mouse erythroleukemic cells, we purified FOG-1 protein complexes from crude nuclear extracts and identified FOG-1 interacting proteins by mass spectrometry. Apart from the known multi-protein complexes that FOG-1 recruits into association with GATA-1, such as the nucleosome remodeling domain (NuRD) complex and C-terminal binding protein (CTBP)-containing complex, we identified a large number of novel FOG-1 interacting proteins involved in various biological processes such as DNA methylation (e.g. DNMT1, Kaiso), chromatin modification (Brd4) and other transcription factors (Myef2). Interestingly, we also found FOG-1 to be co-purifying with several members of the cohesin complex as well as the cohesin-associated protein CTCF. Cohesins are involved in sister chromatid cohesion and along with their functionally associated protein CTCF they have been implicated in the long-range regulation of gene expression. Further validation by streptavidin pulldown assays and immunoprecipitation experiments has confirmed these interactions. These results suggest that FOG-1 may be involved in the formation of looped contacts between target promoters and enhancers (DNA looping) and thus provide FOG-1 with a potential novel role in the regulation of long-range chromatin interactions.

P49 Identification of a critical region within LRRK2 that mediates the interaction with the cell death adaptor protein FADD

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Mutations in the gene encoding leukine-rich repeat kinase 2 (LRRK2) – a large (280 kDa) multidomain protein – are the most common cause of familial Parkinson's Desease (PD).Understanding the mechanisms of mutant LRRK2-induced neurotoxicity can provide greater insight into the pathogenesis of PD. Previous cellular models of LRRK2 neurotoxicity have identified the extrinsic adaptor protein FADD as critical for the induction of apoptotic death in neurons expressing PD-linked LRRK2 mutants. Mutant LRRK2 interacts more strongly with FADD compared to WT, and both proteins form a complex leading to the recruitment and activation of caspase-8. While it is known that the death domain (DD) of FADD interacts with LRRK2, it is not known which region of LRRK2 is necessary for the interaction with FADD. The aim of this study is to map the FADD-interaction domain of LRRK2 with the goal to block the neurotoxic interaction between mutant LRRK2 and the pro-apoptotic protein FADD. Our goal is to determine if specific residues within this domain are required for neurotoxicity induced by mutant LRRK2. We found that there is a critical region within the N-terminal domain of the protein that is able to alter the binding efficiency of LRRK2 to FADD. Moreover we showed that blocking the interaction between mutant LRRK2 and FADD leads to neuronal survival.

P50 Non natural fatty acids binding affinity to bovine β-actoglobulin_Crystallographic and thermodynamic studies.

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Bovine β-lactoglobulin (β-Lg) is a globular protein of 18.4 kDa, consisting of 162 amino acids. It is the major whey protein in bovine milk but it is absent in human milk. Despite intensive studies on biological, chemical, and physical properties of this protein, its biological function still remains unknown. (Le Maux et al. 2012) β-Lg structurally belongs to the lipocalin family, which all members share a common characteristic structural feature with up-and-down eight-stranded β-barrel often called calyx. Central β-barrel makes the hydrophobic scaffold of lipocalin molecule and is primary binding pocket for hydrophobic ligands, such as fatty acids, and hydrophobic vitamins. (Kontopidis, Holt, Sawyer 2004) Among these ligands, fatty acids are the most abundant endogenous ligands of β-Lg (Collini et al. 2003), however this is the first time that non natural fatty acids were studied as ligands for this natural protein.

Thermodynamic and structural studies of β-Lg complexes with no natural fatty acids (tri-, penta-, hepta- and nona-decanoic acid), were conducted. Crystal stuructures of β-Lg complexes with non natural fatty acids were obtained. The interactions of the above mentioned non natural fatty acids with β-Lg were also studied by Isothermal Titration Calorimetry with very promising results.

The β-Lg ability to bind wide spectrum of nature hydrophobic compounds and currently no-natural ligands makes it a promising candidate for utilization as a drug carrier. It could also find application in food industry as a protein that protects hydrophobic molecules from destruction in food processing (Loch et al. 2012).

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P51

Surface Plasmon Resonance in the investigation of protein membrane interactions

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Functional interaction of proteins with the membrane regulate a multitude of cellular functions with therapeutic interest. We have developed and demonstrate state-of-the-art surface plasmon resonance (SPR) technology for real time study of protein membrane interactions. Using ProteOn XPR36 (Bio-Rad), a unique multiplex SPR biosensor, we apply proprietary and customized protocols for liposome tethering on the biosensor chip. The tethered liposomes can form several layers on the chip and are stable under physiological conditions. We demonstrate the feasibility of the technology presenting SPR data from recognition of antibodies to liposome bound antigens, using artificial liposomes.

To further validate the method, we monitored direct binding of recombinant wild type and oncogenic H1047R mutant of phosphoinositide-3-kinase-α (PI3Kα) to liposomes prepared from human colorectal cancer cells. The preliminary data presented here demonstrate the resolving power of SPR methodology in the study of lipid kinases and suggest avenues for the design of novel therapeutics.

P52

The DNMT1 DNA methyltransferase interacts with multiple transcription factors in erythroid cells

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DNA methylation plays important regulatory roles in development as an epigenetic repressive mark, however little is known about the molecular basis of DNA methylation functions in terminal differentiation or how DNA methylation is targeted to specific genes. We used a biotinylation tagging approach to isolate protein complexes for the DNMT1 maintenance DNA methyltransferase from mouse erythroleukemic (MEL) cells, followed by mass spectrometry. We identified novel interactions between DNMT1 and several key hematopoietic transcription factors, such as GATA-1, Gfi-1b, ZBP-89, ZNF-143, FOG-1, and others. These were confirmed by immunoprecipitations which further suggested that DNMT1 forms a core complex with ZBP-89 and ZNF143 and subcomplexes with GATA-1, FOG-1 and Gfi-1b. In order to map the DNMT-1 domains necessary for the interaction with hematopoietic transcription factors, DNMT1 deletion mutants were used to show that the domain responsible for interaction with transcription factors is the 17amino acid PCNA binding domain (PBD) which is responsible for the recruitment of DNMT1 to replication foci. We next used a mutant PBD domain bearing a Q (162) to E point mutation that abrogates the interaction with PCNA, to show that it can still mediate interactions with hematopoietic transcription factors; hence their interaction is unrelated to DNA replication. In order to obtain functional insight as to the DNMT1-transcription factor interactions in erythroid cells, we are currently undertaking a dominant negative approach by overexpressing the PBD-Q162E mutant specifically in the erythroid lineage of transgenic mice.

P53

Thermostability study of analytical grade purified C-Phycocyanin by Differential Scanning Fluorimetry (DSF) under a spectrum of conditions

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C-Phycocyanin (C-PC) is one of the major phycobiliproteins in the cyanobacterium *Spirulina platensis*. It participates in the constitution of molecular complexes that are known as phycobilisomes which are responsible for light-harvesting during the process of photosynthesis. Its distinctive blue color is due to the presence of the chromophores, known as phycobilins, that are covalently attached to the C-PC protein backbone. C-PC demonstrates an absorption maximum at 615-620 nm, while its emission maximum is observed at 647 nm. It consists of two subunits, α and β, that interact and form dimers (αβ), trimers (αβ)3 and hexamers (αβ)6. C-PC is widely used in biotechnology since it displays high stability in a wide range of pH and temperature conditions, while its fluorescence is sustained. Also, low-purity C-PC is applied as a colorant in the food and cosmetics industries. Moreover, antibodies conjugated to C-PC are convenient and useful tools in a variety of immunoassays, such as immunofluorescence, Fluorescence Activated Cell Sorting (FACS) and western blotting, for research and diagnostic purposes. Here, we report the isolation and purification of analytical grade C-PC and the study of its thermal stability under various conditions of ionic strength, pH or detergent concentration, by Differential Scanning Fluorimetry (DSF). Furthermore, we investigated the stability of self-crosslinked C-PC. C-PC exhibited a great stability in a broad range of pH and ionic strength conditions. Our study highlights the compatibility of C-PC with a variety of conditions and its utility as a fluorescent label.

P54

INVESTIGATION OF THE STRUCTURE-ACTIVITY RELATIONSHIP OF e3 UBIQUITIN LIGASES VIA NMR SPECTROSCOPY: MUTATIONS OF aRKADIA RING-H2 DOMAIN

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Protein ubiquitination and subsequent degradation of intracellular proteins plays a crucial role for many cellular functions. Ubiquitination occurs through an enzymatic cascade which includes an ubiquitin- activating enzyme E1, an ubiquitin conjugating enzyme E2 and an ubiquitin ligase E3. E3 ubiquitin ligases are responsible for target substrate recognition and degradation by the 26S proteasome [Inoue Y and Imamura T Cancer Sci 2008, 99, 11]. RING type E3 ligases function through a characteristic domain in their C-terminus. Arkadia is the first example of an E3 ligase that positively regulates TGF–β family signaling through its C-terminal RING domain [Episkopou V et al. PLoS Biol 2007, 5, e67]. The Arkadia RINGs were cloned and expressed in their Zn-loaded form and studied through NMR Spectroscopy [Kandias NG et al. *BBRC* **2009**, 378, 498]. The 3D NMR solution structure of Arkadia RING was determined and deposited in PDB (2KIZ). Additionally, NMR-driven titration studies were also performed to probe the interaction interface of Arkadia RING and its partner E2 (UbcH5B) enzyme. The RING-E2 complex was constructed through an NMR-driven docking, too [Chasapis CT et al. *Proteins* **2012**, 80, 1484]. In order to investigate the role of Zn ion in the structure and function of Arkadia, two mutations were designed, H962C and H965C. Further research is currently taking place to explore their structure and interaction with E2 enzyme via NMR spectroscopy.

P55

Molecular Analysis of CDKL5 gene in patients with Rett-like features: a novel mutation in the carboxy-terminal region

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Mutations in the Cyclin-Dependent Kinase-Like 5 gene (*CDKL5*) located in the Xp22 region have been shown to cause a subset of atypical Rett syndrome with infantile spasms or early seizures starting in the first postnatal months. For the first time in Greece, mutation screening of *CDKL5* was performed in 20 female and 5 male patients, referred for infantile spasms, who were previously tested negative for methyl CpG-binding protein 2 gene (*MECP2*) mutations. All coding exons (2-21) and intron-exon boundaries of *CDKL5* gene were screened for mutations using the simple Enzymatic Cleavage Mismatched Analysis (ECMA), followed by direct sequencing. One novel frameshift mutation (c.2530delC; p.H844IfsX19) was disclosed and predicted to affect the carboxy-terminal region of the protein. The patient, a girl aged 10 years old, presented psychomotor retardation and epileptic spasms beginning at the second month of life. Subsequent studies in both parents and in 50 chromosomes from normal subjects failed to detect the c.2530delC mutation indicating that it is probably *de novo* and disease-causative. The novel mutation was directly submitted in the RettBASE (IRSF MECP2 Variation Database) and LOVD databases. To the best of our knowledge, this is the first time that *CDKL5* gene was studied in patients with Rett syndrome-like phenotypes and epileptic spasms with early onset in Greece. The clinical sensitivity of *CDKL5* mutation screening in female patients with Rett-like features who were negative for *MECP2* mutations was 5% (1/20), a rate slightly lower than those reported by other studies (8%) probably because of their larger sample sizes.

P56

Genetic analysis of the ACVRL1 gene in a large Greek family with HHT

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Hereditary Hemorrhagic Telangiectasia (HHT) or Rendu-Osler-Weber syndrome is a disease caused by abnormal blood vessel formation in the skin and mucosal linings of the nose and gastrointestinal tract leading to nosebleeds, skin lesions and chronic digestive tract bleeding. HHT is a heterogeneous autosomal dominant disease with a prevalence of 1:5000, and it is caused by mutations in three genes namely ENG, ACVRL1 and MADH4. Mutations in each gene lead to different types of HHT, with ACVRL1 mutations leading to HHT2 type. We have previously reported 3 families of Greek origin with HHT. Here we report the genetic analysis of ACVRL1 in 5 additional families including a large one. Genomic DNA was isolated from peripheral blood samples and PCR tests were performed on all 9 coding exons. PCR products were then purified and subjected to automated sequencing. We have identified a novel mutation in exon 7 of the ACVRL1 gene in our large family of 6 generations, plus in 3 other families. The mutation segregates with the disease in all affected individuals but is not present in the unaffected ones as confirmed by RFLP analysis. This study aims to give an indication of the prevalence of ACVRL1 mutations in the Greek population, and will assist in providing a pathogenic mutation profile of this gene.

P57

ADAMTSs in colorectal cancer

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Members of the ADAMTSs family are extracellular multifunctional proteases that degrade proteoglycans, collagens and other [glycol]proteins and thereby have the potential to regulate cellular functions such as adhesion, proliferation and migration. In this study, the expression status of ADAMTS-1, -4, -5, -8, -12 and ADAMTS-20, in both mRNA and protein levels, was investigated in colorectal tumors (N=22) of different cancer stage and anatomic site and in three cell lines of different aggressiveness. The results showed a positive correlation between *ADAMTS-4* and *-5* expression and cancer progress, in contrast with the anti-angiogenic members of the family, *ADAMTS-1, -8* and *-20,* which were found to be down-regulated. *ADAMTS-4* and *-5* seemed to be differentially regulated by serum components. Furthermore, immunohistochemical analysis revealed different localization pattern between ADAMTS-4 and -5, suggesting their different contribution in tumor progression.

P58

Correlation of TACR3 gene polymorphism with Rosacea

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Rosacea is a persistent inflammatory skin condition of the middle face, whose pathogenesis is possibly mediated by a model of neurogenic inflammation. Substance P and/or bradykinin may play a decisive role in increasing blood flow and/or vascular density in rosacea. Neurokinin B-elevated plasma levels are associated with increasing blood flow to the uroplacental unit in pre-eclampsia-associated pregnancies. We chose to study polymorphism rs373631C/G at 5'ut of the neurokinin B receptor (TACR3) because this gene is involved both in the pathophysiology of Parkinson's disease that frequently coexists with rosacea (1) and in the hot flushes experienced by menopausal women (2) similar to those in rosacea.

Genotyping of 128 rosacea patients and 121 healthy controls matched by gender and age, showed statistically significant higher frequency of G/G or C/G genotype (29.5% vs 12.4%) (p=0.006) and G allele (20% vs. 16%) (p=0.004) in patients with the papulopostular (PP) form of rosacea compared to controls. These differences were contributed mainly by male patients with PP rosacea (35.7% vs 13.0% and 21.4% vs 7.0%) (p=0.021 and 0.008 respectively). Marginal differences (p=0.052) in genotypes were observed in erythemato-telangiectatic (ET) rosacea.

This indicates involvement of TACR3 in rosacea. Possibly G allele carriers may provide a genetic predisposition that might facilitate the evolving of chronic persistent stasis erythema to PP rosacea but not to the established ET form and this is more evident in male patients.

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P59 Overexpression of GPC6 and TMEM132D correlates with CD8+ T – lymphocyte infiltration and increased survival in early stage ovarian cancer

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Ovarian cancer is one of the most common types of cancer and the major cause of death from gynecological malignancies. The majority of patients are diagnosed at advanced stage. The high mortality rates are due to the absence of clear symptoms at the early stages of the disease and the lack of a single biomarker with high specificity and sensitivity for prognosis and diagnosis. It is well established that dense infiltration of CD8+ T – lymphocytes at the tumor sites correlates with increased survival rates and favorable clinical outcome. Still, the genes and mechanisms that orchestrate this infiltration remain vague. In order to study the gene expression profile of early stage ovarian tumors enriched in CD8+ T – lymphocytes, we applied a Differential Display approach and identified 128 overexpressed genes. We further evaluated the expression of two genes encoding transmembrane proteins, Glypican 6 (GPC6) and Transmembrane protein 123D (TMEM132D). We validated our results with qPCR in a larger number of patients with early stage of ovarian cancer. The expression of both genes correlated with the expression levels of CD8A, a marker of T – cell infiltration ($p<0.01$ and $p<0.0001$). More importantly, the expression levels of GPC6 and TMEM132D also correlated with overall patient survival. In particular, high levels of both GPC6 and TMEM132D were related with a better overall survival ($p=0.028$). Thus, GPC6 and TMEM132D could serve as potent markers for prognosis and CD8+ T – cell infiltration in early stage ovarian cancer.

P60 Caprine PrPC variants with protective effects against scrapie, as evaluated on a cell culture model of the disease

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Scrapie is a fatal neurodegenerative disease of sheep and goats, characterized by the conformational conversion of the normal, a-helical-rich prion protein -termed PrP^{C}- to its β-sheet-rich isoform, designated PrP^{Sc}. Studies on sheep scrapie have linked ovine *prnp* gene polymorphisms with resistance or susceptibility to the disease. These polymorphisms encode PrP^{C} variants displaying differential conversion efficiencies upon interaction with PrP^{Sc}. Even though sheep scrapie has been extensively studied, goat scrapie has only recently gained much attention. Polymorphisms at codons 142, 143, 154, 146, 211 and 222 of the caprine *prnp* gene have been associated with different degrees of protection. As in the case of sheep, it is supposed that these polymorphisms result in PrP^{C} variants that are less prone to conversion following interaction with PrP^{Sc}. Here, we report preliminary results on the differential conversion efficiencies of caprine PrP^{C} variants in an *in vitro* scrapie model of murine neuroblastoma cells, persistently infected with the 22L scrapie strain (22L-scN2a cells). Our results show that PrP^{C} variants harbouring the polymorphisms *M142*, *R143, H154*, *D146*, *S146*, *Q211* and *K222* exhibit lower conversion efficiencies to PrP^{Sc} compared to the wild type, after expression in the used cell line and interaction with the endogenous, murine PrP^{Sc}, thus confirming their protective role. Among the tested variants, *S146* displayed very low conversion efficiency, suggesting strong protection. This is in line with previous studies of this variant in field and cell-free conversion systems. To our knowledge, this is the first study of caprine PrP variants in a cell system.

P61

Development of Cell Based Assays for the detection of autoantibodies against AChR, MuSK and LRP4, the main antigens in Myasthenia Gravis

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Myasthenia gravis (MG) is an autoimmune disease characterized by a defect in synaptic transmission at the neuromuscular junction leading to fluctuating muscle weakness and fatigue. The main antigens of MG are nicotinic acetylcholine receptor (AChR) and muscle specific kinase (MuSK), justifying approximately 80-85% of MG cases. Recently, autoantibodies against the low-density lipoprotein receptor-related protein 4 (LRP4) have been identified in several double-seronegative MG patients (dSN-MG, without detectable AChR and MuSK antibodies) justifying only a portion of dSN-MG. However, the SN-MG still presents a serious gap in MG diagnosis and understanding. Our aim is the development of new, more sensitive, assays to be used routinely in the diagnosis of MG for the reliable and efficient detection of antibodies against of these antigens. We have developed cell based assays (CBA) based on human AChR clusters, MuSK and LRP4 expressed in HEK293 cells. So far with these CBAs we have found 6.9%, 23% and 18.7% of dSN-MG patient sera being positive for AChR clusters, MuSK and LRP4 respectively. Interestingly, we found that double positive sera, namely $LRP4^+/AChR^+$ (7.5%) or $LRP4^+/MuSK^+$ (18.2%) are more frequent, compared to $AChR^+/MuSK^+$ double positives as described in the literature. Moreover it is worth noting that we found a few cases of triple positive sera. The improved sensitivity of these diagnostic assays (CBA) seems to rely on the fact that the proteins on their cell membrane environment are in native form.

P62

RAD51D germline mutations in Greek ovarian cancer patients.

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Ovarian carcinoma is the most common cause of cancer death from gynaecologic tumors mainly attributed to the advanced stage of disease at diagnosis. There is an underlying genetic factor for approximately 25% of the ovarian cancer cases diagnosed. Germline mutations in the *BRCA1* and *BRCA2* genes contribute to approximately 18% of hereditary ovarian cancers, while 1% is associated with deleterious mutations in MisMatch Repair (MMR) genes and 5% is associated with other genes, like *PALB2, RAD51C* and *BRIP1.* Studies have shown that ~0.9% of *BRCA1* and *BRCA2*-negative ovarian cancer cases carry loss-of function mutations in *RAD51D* gene. Interestingly, there is no evident association between *RAD51D* mutations and breast cancer predisposition. Our aim was to identify the prevalence of *RAD51D* mutations in Greek ovarian cancer patients. We thus sequenced the full coding sequence and intron-exon boundaries of *RAD51D* in 231 sporadic and 54 familial cases that were previously found negative for the most frequent *BRCA1* mutations. We identified one deleterious mutation and two probably damaging unclassified variants. We also identified 6 non-pathogenic polymorphisms.

This is the first report of deleterious *RAD51D* mutations in women diagnosed with ovarian cancer in Greece. Although these mutations are relatively rare, individuals confer a high relative risk of developing ovarian cancer, which is estimated to be 6.30.

P63

Kallikrein-related protease 6 (KLK6) in colon cancer: mRNA expression and clinical relevance

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Colon cancer is a complex disease and the tumor markers used for the its diagnosis have a limited role due to lack of specificity and sensitivity. As a consequence, new biomarkers are needed. Human tissue kallikrein-related peptidase 6 (KLK6) belongs to the human kallikrein family of genes and is a serine protease. It is deregulated in human malignancies and plays important role in cell growth regulation, angiogenesis, invasion and metastasis. So far small clinical study suggests that KLK6 mRNA expression might correlate to poor prognosis of colon cancer.

In the present study *KLK6* mRNA expression was evaluated in 248 colon tissues (83 adenomas, and 99 cancer tissues for 66 of which paired normal colonic mucosa was also available) by real time qPCR using TaqMan probes. Statistical analysis of the results was performed.

KLK6 mRNA expression was significantly ($p<0.001$) higher in cancerous specimens compared to their non-cancerous counterparts, whereas adenomas showed the highest *KLK6* mRNA expression ($p=0.001$) among all colon tissue types examined. High *KLK6* mRNA expression was significantly associated with more advanced TNM tumor stage (III or IV) ($p=0.012$) as well as with higher tumor grade (III or IV) ($p=0.019$). ROC analysis illustrated that *KLK6* gene expression has a strong discriminatory value between non-cancerous and adenomatous colon tissues (AUC=0.888; 95% CI=0.82 – 0.95; $p<0.001$) and a fair discriminatory value between cancerous and non-cancerous tissues (AUC=0.774; 95% CI=0.70 – 0.84; $p<0.001$). Kaplan-Meier survival curves demonstrated that high *KLK6* mRNA expression was significantly associated with shorter Disease-free ($p=0.009$) and Overall survival ($p=0.001$) of colon cancer patients.

P64

Gelatinases and Hyaluronidases in serum of colorectal cancer patients

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Gelatinases and Hyaluronidases are extracellular enzymes, being present in normal tissues, and their amounts altered in pathologic states. Gelatinases (MMP-2 and MMP-9) are metalloproteinases secreted from cells in latent forms being activated under well-regulated conditions; their activity is regulated by TIMPs. Hyaluronidases are enzymes acting mainly on hyaluronan. Four hyaluronidases are well studied, hyal-1, hyal-2, hyal-3 and PH-20. MMP-2, MMP-9 and hyal-1 are present in serum and their activity is altered in various diseases, as well as in cancer. In the present study, semi-quantitative zymographies were applied to detect MMP-2, MMP-9 and hyal-1 in serum of healthy donors and colorectal cancer patients, before and after surgery, in three months interval for up to one year. The results indicated the presence of only latent gelatinases' forms and of hyal-1, in both cancer and healthy serum. The levels of both proMMP-2 and proMMP-9 were higher in serum of cancer patients before surgery compared to that of healthy donors. On the other hand, hyal-1 activity did not show significant differences. The enzymes behaved differently during the follow-up period, showing a site- and stage-relation. The obtained results suggested that proMMP-9 in serum of colorectal cancer patients might be examined as a biochemical marker for cancer recurrence or metastasis.

P65 DNA polymorphism in pharmacologic treatment of schizophrenia

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Schizophrenia is a severe and common psychiatric disorder afflicting 1% of the world population. A role of many neurotransmitter receptors in schizophrenia was suggested by an association with several polymorphisms located in their coding regions. In Greek population, a significant association is clearly determined between the HTR2 genetic polymorphism and the presence of schizophrenic disorder, manifested as increased risk of schizophrenia for carriers of the T-102 allele. In this study we examined the T-102C and A-206G transitions in the 5-HTR2a and DRD3 receptor genes, respectively, in 60 patients, in relation to the disease state and their response rate in pharmaceutical treatment, using PCR and RFLP analysis for the above polymorphisms. Our results showed that the majority of patients were heterozygotic for either of receptor genes. Better response in the treatment was obtained for patients exhibiting on the same time heterozygotic profile for DRD3 and homozygotic profile for mutated HTR2 receptor genes.

P66 Down-regulation of Kallikrein-related peptidase 7 (KLK7) in HCT116 colon cancer cells: Impact on invasiveness and proteomic analysis

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Kallikrein-related peptidases are deregulated in colon cancer. *KLK7* is up-regulated in colon cancer on the mRNA and protein levels, but its function in the progression of this type of cancer remains unclear.

Small interfering RNA (siRNA) targeting *KLK7* was transfected into HCT116 colon cancer cells, which highly express *KLK7*. KLK7 mRNA and protein levels were examined by real time qPCR and Western blot, respectively. The Matrigel™ invasion assay was implemented to assess cell invasion. LC-MS/MS proteomic analysis was applied in order to detect differentially expressed proteins between *KLK7*-silenced and non-silenced colon cancer cells. Statistical analysis was performed to evaluate the significance of the findings.

KLK7 expression was significantly down-regulated (65-80% knockdown) at the mRNA and protein levels in HCT116 cells transfected with *KLK7* siRNA, and the invasive capacity of cells was significantly decreased ($p=0.016$). Proteomic analysis identified 1895 proteins, forty-five of which showed very significant ($p<0.01$) differential expression between *KLK7*-silenced and non-silenced colon cancer cells.

Proteins involved in cell survival as well as in intracellular protein transport were differentially expressed between the two groups. KLK7 peptidase may play an important role in colon cancer invasion and could be a potential target for anticancer therapy.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

P67 Kallikrein-related peptidase 5 (KLK5) in colon cancer: gene expression and clinical evaluation

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Kallikrein-related peptidases (KLKs) are a subgroup of serine proteases located on chromosome 19q13.3. Most members of the Kallikrein-related peptidase family have been extensively studied as potential biomarkers for several carcinomas and other diseases. *KLK5* (*HSCTE*) was originally identified from a keratinocyte library, and its enzyme was purified from stratum corneum of human skin. *KLK5* has been shown to be differentially expressed in a variety of endocrine tumors, but is not as yet examined in colon cancer.

In this study, we assessed the expression status of *KLK5* in 170 colon tissues as well as in 70 adenomas and their paired normal colonic mucosa, by real time qPCR using TaqMan probes.

It was shown that *KLK5* positive expression is associated with patients nodal status (p=0.022) and with tumor histological grade (p=0.033). Cox univariate analysis revealed that *KLK5* positive expression is associated with disease-free survival (p=0.028) and overall survival of patients (p=0.048). Kaplan-Meyer survival models showed that patients with positive *KLK5* have lower disease-free survival (p=0.009) and overall survival (p=0.019). ROC analysis demonstrated that *KLK5* expression had significant discriminatory value between cancer and adenoma tissues (AUC 0.77; 95% CI=0.69-0.85, p=0.03

P68 Development of a multiplex real-time PCR method for antiviral resistance mutation testing of HCMV strains after treatment with ganciclovir and valganciclovir

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Development of drug resistance due to widespread use of ganciclovir (GCV) to treat human cytomegalovirus (HCMV) infections consists a drawback in the treatment of immunocompromised patients. To date, in 85-95% of patients, GCV resistance results from UL97 kinase mutations and there are still no commercial diagnostic methods available for mutation identification.

Our aim was to develop a genotypic resistance testing method for detection of six specific UL97 mutations known to be incorporated in 80% of GCV-resistant clinical strains: M460V/I, H520Q, A594V, L595S, C603W and C607Y. We have set up and further optimized a method comprising three multiplex, real-time PCR protocols (*Göhring et al, 2006)* for simultaneous detection of codons 460/520, codons 594/595 and codons 603/607 using hybridization probes labeled with different fluorescent dyes. Mutations were detected through the significant decrease in the melting temperature (Tm), resulting from the unstable binding of the probes with the mutated sequence. Laboratory non-resistant strain AD169 was used as positive control to determine optimum $MgCl_2$, primer and probe concentrations as well as, annealing temperatures and extension times. To evaluate the assay's sensitivity the WHO 1st International Standard for HCMV was employed. In preliminary experiments, six selected clinical samples from transplanted, immunocompromised patients showing resistance in GCV treatment were examined and in 50% of them one resistance mutation was identified. Furthermore, this method enabled semi-quantitative analysis in case of mixed virus populations of resistant and non-resistant strains in clinical samples.

Overall, we have developed a multiplex method to detect multiple HCMV drug resistance mutations rapidly and simultaneously with the aim to promote antiviral resistance studies in the field of HCMV diagnostic testing in Greece.

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P69 Antimicrobial resistance of Helicobacter pylori (Hp) clinical isolates from Greek adult patients. Detection of point mutations associated with macrolide and quinolone resistance

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Antibiotic resistance is a major factor affecting the efficacy of current eradication therapies against *Helicobacter pylori (Hp)* infection, therefore monitoring antibiotic susceptibility of clinical isolates towards the most frequently used antibiotics is imperative.

Strains isolated during the last 2 years (N=110) from symptomatic adult patients (52.3±14.3yo), who had not received any prior eradication therapy or proton pump inhibitors, were evaluated for antibiotic resistance by E-test, against amoxicillin, clarithromycin, tetracycline, metronidazole and levofloxacin. Minimum Inhibitory Concentration breakpoints were set as follows: amoxicillin (>0.5mg/L), clarithromycin (>0.5mg/L), levofloxacin (>0.5mg/L), tetracycline (>1mg/L) and metronidazole (>8mg/L). The type of mutations associated with clarithromycin and levofloxacin resistance were determined by Real-Time PCR and by amplification and direct sequencing, respectively.

No resistance to amoxicillin or tetracycline was detected. However, primary resistance levels to metronidazole, clarithromycin and levofloxacin were determined at 37.3% (41/110), 24.5% (27/110) and 15.5% (17/110), respectively. The predominant mutations in the 23S rRNA gene conferring resistance to clarithromycin were found to be A2143G and A2142G. In addition, mutations correlated with levofloxacin resistance were Asn87Lys and Asp91Asn in the gyrase A gene. The high primary resistance levels to metronidazole, levofloxacin and especially to clarithromycin, observed within our symptomatic adult population, raise questions about the effectiveness of eradication schemes in the empirical treatment of *Hp* infection in Southern Greece. They also point towards the importance of performing antibiotic susceptibility testing, prior to prescribing an effective eradication therapy, especially following initial failure to clear the infection.

P70 Establishment of a Next Generation Sequencing strategy to identify novel mutations and develop a comprehensive and accurate protocol for the genetic diagnosis & prognosis of familial cardiomyopathies

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Heart Failure due to Cardiovascular Disease is the most prevalent cause of death in the Western World. Cardiomyopathies are commonly inherited disorders of the myocardium and are mainly linked to mutations in genes encoding proteins of the cardiomyocyte cytoskeleton, intercalated discs, contractile apparatus and Ca^{+2} homeostasis. Thus far, genetic diagnostics of Cardiomyopathies are limited by several factors allowing a large number of mutations and more importantly combinations of mutations that give rise to variable disease phenotypes to remain unidentified. To address the need for the development of a method for the simultaneous detection of as many as possible cardiomyopathy causing mutations in known and new-candidate genes, we are employing a deep sequencing protocol. It involves the systematical screening of DNA from patients with different forms of cardiomyopathy for the identification of mutations in a set of 142 selected known and candidate genes that potentially underlie cardiomyopathies. This is performed with the Sequence Capture technology that allows the robust enrichment and analysis of a subset of genes within a genome. Two different Sequence Capture probeset designs have been constructed aiming to the comprehensive capture and coverage of all exons and exon flanking regions of the 142 underlying genes. So far, sequencing of samples from patients with known mutations have captured both the known mutations and also identified several other potential modifiers whose combination probably reflects the different severity of the disease phenotype in different individuals. Sequencing more DNA samples from a large pool of patients will identify novel mutations and perhaps more importantly, provide the necessary robustness to identify such combinations of mutations, ultimately allowing the more effective and diagnosis and prognosis.

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P71

Investigating the Genetic Architecture of Diabetes Mellitus Type 2 on a worldwide level: Implications for future research

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Diabetes mellitus type 2 (T2DM) is estimated to affect 366 million people worldwide and is currently the fifth leading cause of death worldwide. T2DM contributes to the development of a variety of serious complications increasing the public health cost that is associated to the disease. Its prevalence is predicted to have risen by 50% by 2030. High concordance rate in monozygotic twins and first-degree relatives suggest a strong genetic component for the disease. Genome wide association studies (GWAS) performed on type 2 diabetes patients have implicated a large number of single nucleotide polymorphisms (SNPs) in the etiology of T2DM. Here, we investigate the patterns of T2DM associated SNPs across worldwide populations, aiming to understand possible differences in T2DM susceptibility around the world. We analyzed T2DM genes identified through GWAS in 11 populations from four continental regions (available through HapMap 3). Allele frequency as well as haplotype and linkage disequilibrium patterns were investigated. We reveal significant differences across studied T2DM susceptibility variants in different geographic regions which could potentially translate in increased risk for T2DM in specific populations. Our results can contribute to the guidance of worldwide investigations, providing valuable insight into the apparently diverse genetic architecture of T2DM around the world. This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES. Investing in knowledge society through the European Social Fund (MIS 380273).

P72

Individualizing clozapine and risperidone treatment for schizophrenia patients

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Schizophrenia is a severe disorder that significantly affects the quality of life and total functioning of patients and their caregivers. Clozapine is the first atypical antipsychotic with fewer adverse effects and established efficacy. As a rule of thumb, risperidone is one of the most reliable and effective antipsychotics for newly and chronic schizophrenics. Pharmacogenetic studies have identified polymorphisms of candidate genes that seem to be important in the way a patient responds to treatment. The recent progress made in Pharmacogenomics will improve the quality of treatment, since drug doses will be tailored to the special needs of each patient. In this article, we review the available literature attempting to delineate the role of genomic variations in clozapine and risperidone response in schizophrenic patients of various ethnicities. We conclude that pharmacogenomics for these two drugs is still not ready for implementation in the clinic.

P73 A large multi-center pharmacogenetic study of ABCB1 gene polymorphisms and cyclosporine treatment response in patients with psoriasis in the Greek population

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Psoriasis is a chronic, inflammatory skin disease that affects 2-3% of the population worldwide, causing significant morbidity and high financial burden. There are several therapeutic approaches to its treatment, with immunosuppressive drugs such as cyclosporine considered to be first-line systemic therapies for moderate to severe forms of psoriasis. However, patients exhibit heterogeneity in their response to therapy, which could be due to genetic factors.

The aim of the present study, which is based on a Greek multi-centre collaboration, was to target the *ABCB1* gene, which encodes for P-glycoprotein, by selecting functional polymorphisms covering most of the coding regions of the gene locus that could influence the absorption and disposition of P-glycoprotein substrate drugs like cyclosporine. In detail, T-129C (rs3213619), G1199A (rs2229109), C1236T (rs1128503), G2677T (rs2032582) and C3435T (rs1045642) polymorphisms were selected as candidate markers of response to cyclosporine treatment after 3 months of therapy and genotyped in 84 psoriasis patients under cyclosporine therapy. Fifty-two patients (62%) were defined as responders (ΔPASI ≥75%) and thirty-two (38%) as non-responders (ΔPASI ≤50%) to cyclosporine treatment. All SNPs studied complied with Hardy-Weinberg equilibrium distribution and single-SNP and haplotype analyses were performed in order to access responsiveness to treatment with cyclosporine.

This study is the first in the field of the pharmacogenetics of cyclosporine in psoriasis whose results merit further exploitation and validation in larger independent cohorts constructed by multi-center as well as multi-national collaborations in order to identify efficient biomarkers in the prognosis of psoriasis patients' response to cyclosporine.

P74 Analysis of CYP46A1 gene SNP in a Greek population with Age-related Macular Degeneration

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Age-related Macular Degeneration (AMD) is the leading cause of severe visual loss among people over 60 years old. The lack of a broadly effective treatment for AMD underscores the need to identify causative biomarkers that could serve as preventive targets. Local and systemic complement activation occurs in individuals with AMD. Thus far, a large number of independent genetic studies have consistently confirmed the association of AMD with risk or protective variants in genes coding for complement proteins.

The primary goal of the present study was to elucidate whether a single nucleotide polymorphism (SNP), rs754203, which is detected in the cholesterol-24S-hydroxylase gene (CYP46A1), is a major genetic determinant of AMD in a Greek population. The frequency of polymorphism rs754203 in DNA samples (83 patients and 112 healthy Greek individuals) was detected by PCR, fragmentation with restriction enzyme (Sma I) and DNA sequencing. The statistical analysis of the results revealed minor differences in the frequency of rs754203 polymorphism in the groups of patients and healthy individuals, and considerable differences in the patient subgroups, particularly in association with the presence of other polymorphisms in complement genes. In conclusion, while polymorphism rs754203 does not constitute a susceptibility marker for the appearance of AMD, it seems to relate with the progress and magnitude of the disease and there is a possibility that it may act cooperatively with other complement gene polymorphisms.

P75

Gene expression and subcellular localization analysis of HURP in gynecological and gastroenterological tumors

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Aberrant expression of mitotic genes can lead to genomic instability and aneuploidy, a hallmark for cancer. HURP (Hepatoma Up Regulated Proteins) is a tightly cell cycle regulated mitotic protein and its elevated **expression** has been associated with Hepatocellular Carcinoma (HCC) and urinary bladder Transitional-Cell Carcinoma (TCC). Functionally HURP is a Microtubule Associated Protein (MAP) that stabilizes microtubules and is required for the formation of the mitotic spindle apparatus and the proper progression of cell division. Very little is known though, about the role of HURP in other types of human cancer and its possible clinical significance in cancer prognosis or treatment.
To this end we investigate mRNA and proteins levels of HURP in resected human lesions of gynecological and gastroenterological origin, like ovarian and endometrial cancer as well as colon and pancreatic cancer, respectively.
For this study we performed gene expression for HURP using Quantitative Real Time PCR (RT-qPCR). We also examined the abundance and the localization of HURP by immunofluorescence labeling of formalin-fixed paraffin-embedded (FFPE) tissue and subsequent confocal microscopy imaging.
We observed elevated expression of HURP in all the analyzed tumors types, showing higher overexpression in ovarian cancer samples. We also detected overexpressed protein by tissue immunofluorescence analysis against HURP and in some cases we identified differential subcellular localization in tumors cells compared to the normal adjacent tissue.
In conclusion, our findings support the idea that HURP acts as an oncogene in tumorigenesis and underlie the possible significance of the deregulation of HURP localization in cancer.

P76

A MOLECULAR CYTOGENETIC STUDY OF MANTLE CELL LYMPHOMA (MCL) AT DIAGNOSIS AND FOLLOW-UP: EVIDENCE FOR A "TEMPORALLY ORDERED" CYTOGENETIC EVOLUTION?

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BACKGROUND: Apart from t(11;14)(q13;q32), MCL is also characterized by other nonrandom cytogenetic findings. These additional aberrations are well studied at diagnosis and believed to represent clonal evolution during lymphomagenesis, but little is known about karyotypic changes during the course of the disease.

METHODS: The study included 66 patients with MCL. In all cases, an interphase FISH assay was performed for the detection of t(11;14), +12, del(13q14), and abnormalities of ATM, p53, p16, TEL, c-MYC and BCL6 genes. In 28 cases, the same FISH screening was repeated at least once during the course of the disease.

RESULTS: The most frequent additional findings at diagnosis were ATM deletion and del(13q14), followed by p16 deletion, p53 deletion and +12, duplication of the CCND1/IGH fusion gene and BCL6 triplication. 22 of the 28 cases studied at follow-up showed karyotypic evolution, with acquisition of p16 deletion, TEL deletion, duplication of the CCND1/IGH fusion, p53 deletion, and c-MYC amplification. Interestingly, new BCL6 aberrations were seen in 6 cases (at the 3rd/4th repetition of the screening) and the longest survival after detection was 3 months.

CONCLUSIONS: In most cases of MCL clonal evolution occurs during the course of the disease, with the acquisition of multiple additional chromosomal lesions. Some of the aberrations are most commonly present at diagnosis, while different aberrations appear more often or even exclusively on follow-up. From the clinical point of view, the most informative finding is the overrepresentation of the BCL6 gene, apparently associated with aggressive behavior and perhaps the terminal stage of MCL.

P77

TEACHING APPROACHES TO MOLECULAR GENETICS (MODELS SIMULATIONS, METHOD origami, Theatrical Performance)

Lyras John

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The most a lot of knowledge in biology listed in tiny and molecular level. The curriculum identifies as a first order to familiarize students with the structure and levels of organization of living beings. Thus, required the use of appropriate teaching aids for understanding abstruse concepts for pupils to learn the secrets hidden in the life of organizations.
In triangle Pupil - Teacher - Teaching, you need:
The proper design of teaching materials, the clear definition of objectives , and answers to questions such as which concepts and in what order they taught and finally perceptualized information to make more meaningful , fun , digestive and assimilative knowledge and learning.
In the course of biology perceptualized of difficult conditions - concepts - functions, quicken the conquest and assimilation of knowledge with the triptych.
I see and I remember
Maker , experiences and Learning
apohti Skills
The iconic figure - compacts million cases categories and physically and spiritually. Models with simplicity, density and briefly give to man to save time and effort. Requires only little time for initial understanding and then implementing and repetition is a pleasant way. The models convert short-term memory to long-term.
* The characteristics of the model are accurate, clear, simple , complete Brief Formulation instruments are the Word , Symbol and Fig. Their advantages are:
* Have a great economy and performance thinking.
* Have no disagreement because of great simplicity and power absorption when taught correctly.
Constitute an effective and powerful dressage mind and multiply capacity .
The advantages of teaching approach method origami (chartodiplotiki) is
* Dr. therapy students and teachers
* Teaches harmony
* Gives skill fingers
* Agility and eyes
* Acquire students high perception
Then the students utilizing the knowledge required to engage in a game of life by experiencing and understanding the microcosm , with theatrical performances .

Graduate biology, University of Patras
Y \ S. My school (Third High School of Patras) , participated in the " Odyssey " and visited senior education officials from the European Union

P78

Modulation of the nuclear pores by the hepatitis virus C

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The nuclear envelope (NE) compartmentalizes eukaryotic cell genomes in the nucleus and enables the uncoupling of nuclear transcription from translation at the cytoplasm. Nuclear pore complexes (NPCs), are supra-molecular protein assemblies embedded in the NE, form highly selective channels and allow nucleo-cytoplasmic transport of mRNA and proteins.
RNA viruses, such as polioviruses, are known to interfere with nucleo-cytoplasmic trafficking by altering NPC composition. This may represent a novel strategy by which cytoplasmic RNA viruses may alter signal transduction in the nucleus and evade host immune defenses. To investigate whether the hepatitis virus C (HCV), a positive sense RNA virus belonging to the Flaviviridae family, targets the NPC, we performed extensive data-mining and meta-analysis of publicly available global microarray expression data from hepatic cells infected with the HCV/JFH1 isolate or on cells stalby expressing the HCV core protein, a major viral transcription modulator. This analysis indicated that the expression of a number of nuclear pore proteins is indeed affected. Surprisingly, some of these nucleoporins are primarily known to constitute the inner core of the nuclear pores than actively participate in the nucleocytoplasmic-trafficking.

We will further present gene expression data based on real-time PCR from HCV/JFH1 infected hepatocytes and core protein-expressing cell lines constructed in our laboratories that confirm changes in the expression of the selected nucleoporins and discuss how NPC conformation and composition might influence HCV replication and pathogenesis.

P79

Development of a novel PCR-based method for rapid assessment of viral nervous necrosis genotypes

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Viral nervous necrosis infections are causing severe problems on aquaculture industry due to ecological and economic impacts. Their causal agent is the viral nervous necrosis virus (VNNV) or Nodavirus. Nodaviruses have been classiffied into four genotypes: striped jack (SJNNV), red-spotted grouper (RGNNV), tiger puffer (TPNNV) and barfin flounder (BFNNV). Different genotypes correlate with differences in viral pathogenicity. Therefore, rational development of effective vaccines and diagnostic reagents requires analysis of the genetic variation. Aim of the present study was to develop a PCR-based methodology for RGNNV and SJNNV genotype assessment in a simple, robust, low cost format, that would facilitate basic research and applications in industry (e.g. by vaccination with genotype-specific vaccines). Genotype discrimination relies on the fact that DNA polymerase requires perfect complementarity between the 3'-end of a primer and the DNA template, for full activity. Degenerate external primers and two genotype specific internal primers were utilized for simultaneous amplification of Nodavirus products in a single PCR. The outer primers have melting temperatures that are 10 ºC higher than those of the internal primers. A first set of cycles produce a long PCR product defined by the outer primers, at high annealing temperature. A second set of cycles is performed in lower annealing temperature and the internal primers amplify short DNA fragments specific for each genotype. Detection is based on the size of the short products. The proposed methodology has been evaluated in terms of specificity and sensitivity and was applied to assess nodavirus genotypes from viruses in fish samples.

The research project is implemented within the framework of the Action «Supporting Postdoctoral Researchers» of the Operational Program "Education and Lifelong Learning" (Action's Beneficiary: General Secretariat for Research and Technology), and is co-financed by the European Social Fund (ESF) and the Greek State.

P80

Engineering Escherichia coli strains that suppress the toxicity of membrane protein overexpression

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Membrane proteins (MPs) include very important protein families, such as G protein-coupled receptors (GPCRs), ion channels, and transporters. Apart from the scientific interest, MPs are extremely important drug targets and, therefore, receive a lot of attention by the pharmaceutical industry. To characterize MPs and to obtain data for the rational design of drugs, large amounts of MPs are required. As the natural abundance of most of MPs is very low, they are produced by overexpression in heterologous hosts. *E. coli* is by far the most widely used expression host for the production of recombinant proteins. However, MPs are notoriously difficult to express as the yields per cell are typically low and MP production is highly toxic to cells leading to low biomass. The aim of the present work is to engineer strains of *Escherichia coli* to withstand the toxicity caused by MP overexpression. The ultimate goal is to use these engineered strains for large-scale production of MPs and to investigate the mechanism behind the observed cytotoxicity. To achieve this, we have generated libraries of mutant bacteria carrying different types of genetic modifications, such as gene overexpressions, mutations and deletions, and used appropriate genetic screens or selections to isolate the desired clones. Using the human GPCR bradykinin receptor 2, a protein with very high overexpression toxicity in *E. coli*, as our model MP, we have identified a number of *E. coli* strains with the ability to suppress cytotoxicity upon MP production. These engineered strains have been partially characterized and a potential physiological role of the identified mutations has been proposed.

P81 Global cellular changes of Lactococcus lactis MG1363 during exposure to different stress stimuli assessed by FT-IR spectroscopy

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During food fermentations lactic acid bacteria are confronted with technological stresses designed to be bacteriostatic or bactericidal for food spoilage microorganisms and foodborne pathogens. *Lactococcus lactis* MG1363 is considered as the model organism for lactic acid bacteria. In this study, we employed this microorganism in order to assess compositional and structural changes at the cellular level during exposure to different stress stimuli including low pH, high NaCl concentrations, non-optimal temperatures (both high and low) and starvation. The method for studying such changes was FT-IR, a non-destructive physicochemical method that produces the chemical fingerprint of cells. The FT-IR spectra of control and stressed cells were recorded. After second derivative transformation of the four spectral regions that are characterized by the absorption of major cellular constituents like lipids of the cell-membrane, carbohydrates of the cell-wall, proteins etc., principal component analysis (PCA) was used for clustering the data. Spectral features of control cells were found distinct from those of stressed cells. Most importantly, PCA analysis could segregate samples according to the stress condition. Our findings suggest alterations of the global chemical composition of cells during stress adaptation and these alterations may be distinct for each stress condition.

P82 Heterologous expression of the mammalian nucleobase transporter rSNBT1 in the LEXSY Leishmania tarentolae protein expression system

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Experimental systems for expression of membrane proteins from higher eukaryotes often do not support the post-translational maturation and correct membrane targeting of these proteins and have problems of cytotoxicity and low expression levels. In this work, we examine the potential of the LEXSY *Leishmania tarentolae* parasites to heterologously express mammalian membrane transport proteins for structural and functional studies. The *L. tarentolae* system is non pathogenic to humans and used at biosafety S1 conditions, requires simple nutrients for growth in culture, allows efficient *N-glycosylation* of mammalian proteins and displays physical auxotrophy on several amino acids, which facilitates potential structural studies. As a study paradigm for expression in this system we used the rat rSNBT1 nucleobase transporter. This transporter belongs to the NAT/NCS2 family of nucleobase transporters, an evolutionarily conserved and phylogenetically widespread family, with a single known x-ray structure from a distantly related bacterial homologue (UraA) and with a limited number of studied homologues. The rSNBT1 transporter was cloned to the pLexsy-sat vector at the 5' end of the mRFP gene. *L.tarentolae* parasites transfected with rSNBT1-mRFP plasmid were selected by antibiotic treatment. The growth characteristics of the transgenic population were compared to the wild type and the correct plasma membrane localization of the rSNBT1-mRFP was evaluated in transgenic parasites by confocal microscopy. Finally the expression of the correct size hybrid proteins was confirmed by Western Blot in parasite membranes using an anti-mRFP antibody. We currently assess the functional properties of the rSNBT1 as nucleobase transporter in live *L.tarentolae*-rSNBT1-mRFP parasites.

Acknowledgment

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: THALES, Investing in knowledge society through the European Social Fund.

P83 High-throughput screening of metagenomic libraries for the detection of thermostable hydrolases using E. coli expression systems

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Enzymes are used as biocatalysts in a wide range of industrial applications, including the food and paper industry, detergents and drugs. A very limited amount of hydrolases is currently being used in the industry the reason being the enormous cost associated with cooling and reheating materials and water, before adding the enzymes. With the availability of thermostable enzymes a number of new possibilities for industrial processes have emerged. The advantages of conducting biotechnological processes at elevated temperatures are numerous including contamination risk reduction by common mesophiles, increased bioavailability and solubility of organic compounds, increased reaction rates due to a decrease in viscosity and an increase in the diffusion coefficient of substrates. Although thermostable hydrolases have been known for many years, the related research and applications have been limited to cultivated thermophilic microorganisms. Since most microorganisms (>99%) cannot easily be cultivated using standard laboratory techniques, many potentially active enzymes have never been characterized. This is particularly true for thermostable enzymes, since the number of isolated and characterized (hyper) thermophiles is very small. Therefore, the diversity of thermophiles and their encoded enzymes remains largely unexplored. This work aims to screen for a new generation of (hyper) thermostable hydrolases from hot terrestrial environments by using high-throughput screening assays. Towards that purpose metagenomic libraries are introduced into *E. coli* expression systems for the detection of lipase, xylanase and cellulase activity. This approach provides an easy and time efficient way to screen millions of pool-DNA fragments for multiple enzymatic activities without having to identify and cultivate the carrier microorganisms.

P84 Identification and characterization of an extracellular component with siderophore-like properties secreted by the thermophilic bacterium Thermus thermophilus HB8

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Iron is essential for microorganisms that have evolved special high-affinity systems for its acquisition from the environment, which called **sidero-phores** excreted under iron deficiency and bind Fe^{3+}. An extracellular component with **siderophore-like** properties was isolated from *T. thermophilus* HB8 cultures containing sodium alginate. The purified component showed high reactivity, with chrome azurol S (CAS) assay revealing that iron may be responsible for this ability. Qualitative determination of metal ions by several techniques revealed the presence of mainly iron associated with this structure. IR spectrum indicated the existence of a hydroxamate-type metal-binding siderophore. The selectivity of the siderophore was evaluated against a range of physiologically relevant metal ions present as their chloride salts. Only Fe(III) showed a selective recognition for the siderophore. The interaction of the siderophore has been studied by fluorescence spectroscopy titration and the changes in the spectra of the siderophore on its binding to Fe(III) used for their interaction study. The K_{SV} Stern-Volmer constant was for the binding interaction between the siderophore and Fe(III) for n = 1:1 (host:guest, i.e. siderophore:Fe(III)) stoichiometry. Additionally, the values of the association binding constant K (=2.42×10^3 M^{-1}) and the number of binding sites per siderophore n (= 0.95) have been obtained from the Scatchard plot. The molecular mass of the component estimated by gel filtration chromatography, and identified by LC-MS. Siderophores may serve as detoxifier in iron overload diseases and in the treatment of anemias (ß-thalassemia), as MRI imaging agents in diagnostic medical and as templates for novel antibiotics.

P85 Identification of potentially therapeutic compounds that enhance the stability of a carcinogenic p53 variant

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The tumour suppressor protein p53 plays a very important role in protecting the cells from carcinogenesis. In physiological conditions, p53 is activated by DNA damage and other stress signals, and leads to cell cycle arrest and apoptosis of damaged cells. However, in 15-20% of human cancer cases, p53 is destabilised by missense mutations. In this way, the apoptotic activity of p53 is inhibited and thus, uncontrolled proliferation of malignant cells occurs. Y220C is the most common mutation of this type and so, comprises an important target for cancer therapy.

The aim of this work is the discovery of compounds that bind to p53(Y220C), stabilise its structure and restore its physiological activity. We propose a novel method, which contrary to conventional approaches, enables us to quickly and easily test a large library of compounds. Specifically, we have genetically modified Escherichia coli cells in order to: (1) produce a library of ~10.000.000 different compounds and (2) allow the identification of the bioactive molecules through a simple and high-throughput screen that links the stability increase of p53(Y220C) with a selectable phenotype.

Application of the above procedure resulted in the identification of one compound that interacts with p53(Y220C) and increases its stability. The ability of this compound to restore the proapoptotic function of p53(Y220C) is further tested, with the hope of becoming a drug candidate against a broad range of human cancers.

P86 Indications of a ring hydroxylating dioxygenase gene involved in the upper pathway of phenanthrene degradation by Arthrobacter phenanthrenivorans Sphe3

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Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous compounds detected in soils and sediments as a result of both natural and anthropogenic activities. Bacterial aerobic PAH degradation, initiated by the introduction of both atoms of O_2 to the aromatic ring, is catalyzed by ring-hydroxylating dioxygenases (RHOs). In silico study in the genome of *Arthrobacter phenanthrenivorans* Sphe3, isolated from a PAH-contaminated soil in Epirus using phenanthrene as sole source of carbon and energy, revealed the existence of putative genes encoding RHOs. Two pairs, consisting of α and β subunits, are located on pASPHE301 (190 kb) and pASPHE302 (94 kb) plasmids and a single subunit on the chromosome of the Sphe3 genome. To identify proteins involved in the upper pathway of phenanthrene degradation, we conducted a proteome analysis of cells grown on phenanthrene, phthalate (intermediate metabolite of phenanthrene degradation), glucose and glucose plus phenanthrene. For this purpose, an in depth, quantitative 'shotgun' proteomics analysis was performed, using ultra high pressure liquid chromatography combined with high resolution mass spectrometry. Multiple changes in the abundance of some proteins, with respect to phenanthrene or phthalate induction, were observed. Among these, a copy of the terminal subunit of an RHO, located on the pASPHE301 was upregulated ~ 10fold only in phenanthrene-grown cells. This result was also confirmed by RT-qPCR. The *phpdc* gene encoding the above α and β subunits of the RHO was amplified with PCR, subcloned in plasmid pET29c and introduced into *E. coli* DH5a. 3,4 Phenanthrene dihydrodiols were identified indicating the phenanthrene oxidation by the *E. coli* (*pET29-phpdc*) recombinant cells.

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P87 Utilization of wine wastes for the recovery of biological active constituents and ethanol production

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Grapes are among the largest fruit crop in the world, of which ~80% is used for wine making. Wine industries produce large quantities of wastes, which untreated have significant pollution potential due to their high content of non-degradable phenolic substances. However winery wastes could be an alternative source for obtaining natural antioxidants, which are considered completely safe. The objectives of this study were to optimize the extraction of antioxidant compounds in red and white grape pomace and determine their antioxidant activity as well as their content in polyphenolic compounds. The results showed that methanol and ethanol were the best solvents for the extraction of phenolic compounds from red and white wine waste respectively (wine waste mass (d.w.) / solvent volume: 1/5, pH 5.0) and this was concomitant with the high antioxidant activity. The antioxidant activity as well as the total phenolics in red wine waste were found to be slightly higher (86% and 2.84 mg/g respectively) comparing to white wine waste (77% and 2.35 mg/g respectively). In addition, *Saccharomyces cerevisiae* and a mutant strain of *Zymomonas mobilis* CP4, tolerant to sucrose up to 40% (w/v), were used to produce ethanol from wine wastes supplemented with different salts. Both microorganisms grew well and the production of ethanol was promising and to around 110 g/l.

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P88 Evidence of two nitrate/nitrite transporter genes in PAH-degrading bacteria Mycobacterium gilvum Spyr1 and Arthrobacter phenanthrenivorans Sphe3

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Biodegradation of polycyclic aromatic hydrocarbons (PAHs) is an important contributor to bioremediation of polluted soils. Bioaugmentation by addition of cultured microorganisms or biostimulation by nutrient application (N, P) is a viable technology and the best option for restoring long-term contaminated soils. Analysis of bacterial nutrient uptake systems could allow optimization of such interventions. The recent genome sequencing of *Mycobacterium gilvum* Spyr1 and *Arthrobacter phenanthrenivorans* Sphe3, two PAH-degrading soil bacteria isolated from an abandoned formerly industrial area in Epirus and characterized in our laboratory, prompted us to investigate the nitrate/nitrite and other metabolite uptake systems of these bacteria. Since nitrate can serve as an electron acceptor, it might be important for growth under oxygen limitation conditions which develop in contaminated soils.
In silico study revealed that both genomes have two putative nitrate-transporter genes which belong to the Nitrate Nitrite Porter (NNP) family of MFS superfamily. The predicted gene products retain the characteristic nitrate-signature motif of the family and conserved Gly or polar residues of MFS superfamily in the central hydrophilic cavity. The four NNP genes were amplified with PCR, tagged with a biotin-acceptor domain at their C termini, transferred to plasmid pT7-5 under control of the *LacZ* promoter/operator and introduced into a mutant *Escherichia coli* strain defective in all three native transporter genes (*narK*, *narU*, *nirC*). Heterologous expression of the NNP genes in the inner *E. coli* membrane was demonstrated by western blotting. The heterologously expressed NNP genes were found to complement the nitrate-dependent growth of the defective *E. coli* strain.

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P89

Heterologous expression in E.coli, isolation and purification of the recombinant human homeobox transcription factor MEIS1

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MEIS1 (Myeloid Ecotropic Viral Integration Site 1) is a homeobox protein, belonging to the TALE (Three Amino-Acid Loop Extension) family of homeodomain transcription factors. Homeodoamin transcription factors, especially HOX factors, play crucial role in embryogenesis and development. MEIS1 can form homo-dimers but also hetero-dimers with various HOX factors, binds to specific DNA sequences and is responsible for proper hematopoiesis and angiogenesis. Aberrant or over-expression of MEIS1 has been reported in various forms of leukemias. Recently, it has been demonstrated that MEIS1 is also involved in ovarian carginogenesis. Here, we report the heterologous expression and purification of the recombinant human MEIS1. We cloned the open reading frame of MEIS1 in pET Duet vector and transformed *E.coli* BL21DE3 cells. Several conditions of induction and lysis were examined for optimizing protein expression and solubility. By applying Differential Scanning Fluorimetry we studied the stability of the recombinant, affinity-purified MEIS1 in various conditions. Finally, the recombinant MEIS1 was used in functional assays in order to study the role of MEIS1 in the DNA binding and transcription of several genes implicated in oncogenesis.

P90

Molecular characterization of Pseudomonas aeruginosa population in aquatic environments of Greece

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Recent isolation of *Ps. aeruginosa* strains from water samples and subsequent PFGE analysis demonstrated that these strains have a unique genotype. *Ps. aeruginosa* isolates from environmental habitats may also have a unique plylogenetic position. Considering the advantages of MLST, as a typing method that focuses strictly on 7 conserved housekeeping genes, it was chosen for typing and discrimination of *Ps. aeruginosa* strains isolated from water samples from all over Greece. Additional information regarding the resistance and serotypes circulating in these environments is cited. The activity of 11 antibiotics was tested against 31 isolates by the disk diffusion method. Serological typing was applied according to the IATS; MLST was performed as described previously with some alterations concerning the annealing temperatures of the seven housekeeping genes. The control strain PAO1 and one isolate randomly chosen were used to test the proposed protocol and the results were compared with the data on the MLST database. 9% of the isolates exhibited the intrinsic antimicrobial resistance, while 13% presented additional resistant mechanisms, which is a high percentage, considering that these are pilot results depending on a small number of environmental isolates. 22.5% of the isolates were serotypeable and distributed into three serotypes. The amended protocol of MLST seemed to produce allelic profiles for the two isolates tested. Thus this typing method is going to be used in a larger sampling program to obtain more data and to conclude in a reliable phylogenetic analysis for the first time in aquatic environments of Greece

P91

Molecular virological screening of three Greek wastewater treatment plants

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As bacterial indicators generally fail to signal the potential for viral contamination, adenoviruses (AdVs) have been proposed as alternative indicators. AdVs have been shown to be excreted by the populations of all geographical areas and to be the most abundant viruses detected in urban sewage [1-3]. A molecular virological screening study of AdVs was performed in the context of the ARCHIMEDES III research funding program entitled: "Wastewater Reuse-Development of a Public Health Risk Assessment Model". The project aimed to evaluate the microbiological and chemical quality of the influent and effluent of three wastewater treatment plants (WWTPs) in Greece, used as models for the formulation of a risk assessment model on wastewater reuse for irrigation purposes.

Wastewater samples (1 L and 10 L from the WWTPs' inlets and outlets, respectively) were collected monthly (January - September 2013) from the WWTPs of Rio-Patra (PAT), Arachova (ARH) and Livadia (LEV). For the virological analysis, protocols of the environmental virology FP7 project VIROCLIME (http://www.viroclime.org/) were used. The skimmed milk flocculation procedure was applied for virus concentration [4], while the viral molecular detection was based on TaqMan assays previously described [5], and designed to quantify all common human AdVs with high specificity in environmental samples [6].

AdVs were quantified at the inlet of the three WWTPs at 687,8 ±1095,7 (LEV), 494,1±670,9 (ARH), and 95,5 ± 127,6 (PAT) GCs/mL, and the outlet at 90,8±178,3 (LEV), 47,1 ±66,3 (ARH), and 14,6±18,3 GCs/mL (PAT). The percentage of AdV reduction for the three WWTPs was calculated at 87,3% (86,8% (LEV), 90,5% (ARH) and 84,7% (PAT)).

The study demonstrated the feasibility of using AdVs as indicators of WWTP's efficiency, and is expected to support the development of a public health risk assessment model on the use of treated wastewater for irrigation purposes. It will provide public health scientists, authorities, environmentalists and other people dealing with wastewater reuse in Greece the opportunity to discuss the issues of effluent virological quality, since current regulations don't include any viral parameter.

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P92

A1-antitrypsin (SERPIN-A1) in colon cancer: gene expression and clinical relevance

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Colon cancer (CC) remains the third leading cause of worldwide cancer-related death in men and women. Currently available prognostic and/or predictive markers for colon cancer lack specificity and sensitivity. Developing new biomarker for early detection, accurate diagnosis and therapeutic treatment for colon cancer is of great importance in improving the clinical outcome of the disease. Members of the serine protease inhibitor (Serpin) superfamily are found in all branches of life and play an important role in the regulation of enzymes involved in proteolytic cascades. The family includes alpha1-antitrypsin, alpha1-antichymotrypsin, C1 inhibitor, antithrombin and neuroserpin. Alpha1-antitrypsin is the prototypical member of the serine proteinase inhibitor or serpin superfamily of proteins. Kallikrein-related peptidases (KLKs) are involved in proteolytic cascades of different tissues. KLK14, acting via PAR-2 represents an autocrine/paracrine regulator of colon tumorigenesis and alpha1-antitrypsin is a natural inhibitor of KLK14. Therefore its role in regulating the proteolytic cascade in colon tumorigenesis is of great importance. In this study, we examined for first time, using quantitative real time PCR, the expression of alpha1-antitrypsin in 101 colorectal carcinoma tissues for 70 of which normal paired mucosa were also available, as well as in 74 colorectal adenomas. We also clinically evaluated the impact of the results for those patients. Alpha1-antitrypsin expression was found to be significantly associated with TNM stage (p=0.028). Cox proportional hazard regression model using univariate analysis revealed that high status alpha1-antitrypsin expression is a significant factor for disease-free survival (DFS) (p=0.002) and overall survival (OS) (p=0.026) of patients. Kaplan-Meier survival curves demonstrated that alpha1-antitrypsin expression of low status is significantly associated with longer DFS (p=0.001) as well as OS (p=0.021), suggesting that alpha1-antitrypsin gene expression may represent a useful marker of unfavorable prognosis for CC.

P93

Signaling and trafficking of Activin A ligand/receptors

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TGF-β superfamily members comprise the largest family of secreted morphogens, regulating a wide range of biological processes, such as cell proliferation and differentiation, maintenance of pluripotency and angiogenesis. Members of TGF-β superfamily bind to heterotetrameric complexes of type I and type II Ser/Thr kinase receptors and transduce signals through R-SMADs. SMAD2 and SMAD3, oligomerize with SMAD4 and rapidly translocate to the nucleus, promoting the transcription of target genes. Also, SMADs interact with transcriptional coactivators and corepressors that modulate the expression output.

To orchestrate diverse biological functions, signaling is spatially and temporally tightly regulated. Internalization of cargo is carried out by various endocytic pathways, including clathrin-dependent and clathrin-independent pathways. Endocytic vesicles are targeted to early endosomes. Sorting events initiated at this compartment determine the fate of cargo, including signaling propagation, recycling to the plasma membrane or degradation in lysosomes.

The target of our research is to investigate the internalization pathways that Activin A receptors follow both in the absence and the presence of ligand, and therefore the impact of trafficking events on signaling. For that purpose different experimental approaches, such as confocal microscopy, live cell imaging and RNAi, were performed on endothelial cells (HUVECs). Our current effort is to apply this knowledge on human embryonic stem cells (hESCs). Though Activin A is a developmentally important molecule and regulates the expression of the pluripotency factor NANOG very little is known about signaling and trafficking of its ligand/receptor complexes in hESCs.

P94

Transporter sorting and endocytosis: lessons from a model fungus

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We investigated mechanisms operating for transporter down-regulation by endocytosis and/or direct sorting from the Golgi to the vacuole in response to broad range physiological signals, stress or genetic mutations. Out model cargo protein was the extensively studied uric acid-xanthine transporter of the model filamentous fungus *Aspergillus nidulans.* A single arrestin-like protein, ArtA was shown to be essential for HECT-type ($HulA^{Rsp5)}$ dependent ubiquitination and endocytosis of UapA in response to availability of rich nitrogen sources or the presence of excess substrates. Mutational analysis showed that residues 545-563 of the UapA C-terminal region are required for efficient UapA endocytosis, whereas the N-terminal region (residues 2-123) and both PPxY motives are essential for ArtA function. We further showed that ArtA undergoes HulA-dependent ubiquitination at residue Lys343 and that this modification is critical for UapA ubiquitination and endocytosis. Our results extend the model on how arrestins function in order to elicit the acceleration of the turnover of a specific transporter in response to its transport activity.

We subsequently found that specific mutations eliciting misfolding and vacuolar turnover of UapA do so by promoting its direct sorting, probably through the AP-3 trafficking pathway, from the Golgi to the MVB compartment. In that case, an ER/Golgi-resident transmembrane adaptor, called BsdA, was identified to be essential for $HulA^{Rsp5}$-dependent ubiquitination and turnover of UapA. This result reveals a novel type Golgi protein quality control for partially misfolded proteins, which escape the ER-based quality control.

P95

Coupling insect olfactory receptor pharmacology with the search for new mosquito repellents

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Functional studies on insect odorant receptors (ORs), including mosquito ones, are of interest, both because such receptors are members of a novel family of heteromeric ligand-gated ion channels and potential targets for development of new repellents.

In this presentation we will describe the functional characterization of *Anopheles gambiae* ORs by an optical cell-based assay employing lepidopteran insect cells and a Ca^{2+}-biosensor photoprotein as an alternative to the more widely used electrophysiological methods. Besides allowing convenient, robust and quantitative monitoring of receptor responses, this system can be used to investigate interesting pharmacological properties of the receptors that include allosteric modulation, desensitization and antagonism.

P96

Esrogens counterbalance the Ang-II-induced oxidative stress in endothelial cells in vitro

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Estrogens favor a well-tuned cardiovascular system, as indicated by the low incidence of vascular events in premenopausal women. Angiotensin II (Ang-II) is a pro-inflammatory factor, which increases free radicals in inflamed endothelial areas. However, the influence of estrogens on Ang-II-induced oxidative stress has not been fully elucidated.

According to our experimental model, EAy926 endothelial cells were exposed to Ang-II (10^{-9} M) for 2 hours and after they were exposed for 2 more hours in 17-β estradiol (0.5pg/mL). Nitric oxide (NO) levels were determined in culture media, whereas superoxide dismutase (SOD), catalase, endothelial nitric oxide synthase (eNOS) activity, as well as GSH concentration and GSH/GSH+GSSG ratio were measured in cell lysates. Intracellular ROS (reactive oxygen species) levels were also estimated. The statistical analysis was performed using ANOVA test. Endothelial cells treatment with Ang-II led to increased ROS levels ($p<0.05$) and decreased activity of the studied antioxidant response enzymic systems, apart from catalase and GSH/GSH+GSSG ratio that remained unchanged, compared to untreated cells. These effects were reversed by E2. Notably, intracellular ROS content decreased to levels significantly lower ($p<0.05$), compared to the untreated and Ang-II-treated cells. Similarly, the activity of catalase ($p<0.01$), SOD ($p<0.01$), eNOS ($p<0.01$) as well as the amount of released NO ($p<0.05$) and the concentration of GSH ($p<0.05$) increased significantly and returned to control levels or even higher. According to our results we can assume that the short-term action of normal E2 levels, inverse strongly the endothelial redox imbalance, caused by Ang-II, and reinforce the anti-oxidant response mechanisms.

P97

Fungal plasma membrane organization: eisosomal proteins

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Eisosomes are punctated structures underlying the fungal cell membrane. They are furrow-like invaginations of the plasma membrane mediated by a conserved BAR domain present in their constitutive proteins. They were first described in *Saccharomyces cerevisiae* as "portals of endocytosis". Their structural assembly is dependent on the cytosolic eisosomal proteins Pil1p and Lsp1p and on sphingolipid levels, through regulation of Nce102p, Pkh1/2p and Ypk1p proteins (Olivera-Couto and Aguilar 2012). Eisosomal specific proteins are found in all ascomycetes and basidiomycetes (C. Scazzocchio, personal communication). In spite of their striking conservation their function remains elusive, deletion of the cognate genes resulting in usually mild and varied phenotypes in different species.

In *Aspergillus nidulans*, contrary to the Saccharomycotina, the fate of the eisosomal specific proteins PilA, PilB and SurG is developmentally regulated (Vangelatos et al. 2010, Scazzocchio et al. 2011, Athanasopoulos et al. 2013). PilA is a BAR domain (Bin/Rvs/Amphiphysin) protein responsible for membrane binding and curvature maintenance. Introduction of specific amino-acid residue alterations in PilA affects its cellular distribution, resulting in fewer and larger eisosomal assemblies in the membrane. Moreover, they affect the distribution of the *A. nidulans* orthologue of Nce102p in both conidia and young germlings originated from conidiospores. Nce102 in non-germinated conidia shows a plasma membrane-like punctated distribution and colocalises with eisosomes. In conidia-originated germlings, Nce102 is confined to eisosomes, vacuoles and endosomes. Deletion of *nce102* results in cytoplasmic localization of PilA and affects the number of eisosomes at the head of germlings originating from conidiospores.

P98

High glucose induces suppression of insulin signaling in human glomerular podocytes

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Loss of podocytes by apoptosis characterizes the early stages of diabetic nephropathy (DN), while during DN, podocytes also become structurally and functionally compromised. Chronic hyperglycemia may disrupt and/or attenuate signal transduction pathways that promote normal podocyte survival, leading to loss of the permselective renal barrier and proteinuria. The aim of this study was to investigate whether high glucose can influence insulin signaling and survival in immortalized human podocytes (human glomerular epithelial cells: HGEC). Results showed that prolonged exposure of HGEC to increased glucose concentrations resulted in significant inhibition of insulin-induced tyrosine phosphorylation of the insulin receptor (IR), and attenuation of expression levels of insulin receptor substrate-1 and -2 (IRS-1 and IRS-2). These changes were accompanied by impaired activation of the anti-apoptotic signalling protein Akt and annulment of Akt-mediated suppression of the Forkhead family of transcription factors (FoxO) activation. Furthermore, the observed IRS-2/Akt-mediated signaling alterations were relatively irreversible. Additionaly, the potential susceptibility of HGEC to apoptosis was measured by Poly(ADP-ribose) Polymerase (PARP) cleavage and caspase-3 activation. These data suggest that since chronic hyperglycemia impaired insulin survival pathway in cultured podocytes, the investigation of any potential protective mechanisms against apoptosis and loss of the permselective renal barrier is of paramount importance.

P99

Induction of MMP-3/10 (stromelysin-1 and -2) activity in Helicobacter pylori-infected gastric epithelial cells may depend on the phosphorylation of the bacterial CagA oncoprotein

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Following adhesion of *H. pylori* to gastric epithelial cells (AGS), the bacterial protein CagA is translocated intracellularly and is hierarchically phosphorylated by Src and Abl kinases, on repetitive EPIYA tyrosine phosphorylation motifs, which plays a determining role in the induction of a scattering phenotype resembling to "epithelial to mesenchymal transition". In *H. pylori* clinical isolates CagA EPIYA motifs vary namely, EPIYA-*A*:EPIYAKVNK, EPIYA-*B*:EPIYAQVAKK and EPIYA-*C*:EPIYATIDDLG and the number of EPIYA-C repeats has been correlated to oncogenic potential. In this study, we investigated the potential involvement of CagA protein in the activation of stromelysins-1 and -2 (MMP-3/-10) in *H. pylori*-infected AGS. We utilized isogenic *H. pylori* mutants, expressing CagA protein with variable numbers (n=0-3) of functional EPIYA-C and phosphorylation-deficient EPIFA-C motifs, as well as the corresponding *cagA*- and *cagE*-knock out strains, to infect AGS cells *in vitro*. MMP-3/-10-specific transcriptional activation was measured by Reverse Transcriptase quantitative Real Time PCR, at several time points. MMP-3/-10 expression was also determined by western blot analysis at 24h post-infection. A nearly 100-fold increase in MMP-3/-10 was observed in the presence of CagA protein and proportionally to the number of EPIYA-C terminal motifs. On the contrary, CagA phosphorylation-deficient mutants induced only background levels of stromelysins, equal to those observed for the *cagA*- and *cagE*-knock out mutants. CagA-dependent increase in gelatinolytic and caseinolytic enzymatic activity was also detected utilizing zymography. CagA expression contributed to MMP-3/-10 expression in total cell lysates, irrespective of EPIYA phosphorylation, whereas secreted MMP-3/-10 levels were found dependent on CagA EPIYA phosphorylation.

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P100 Influence of the Immunological Effector IFNγ on Neuroblastoma cells

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IFNγ is a cytokine that belongs to type II interferons. It plays a crucial role in innate and adaptive immunity whereas its aberrant expression/activity has been associated with a number of autoimmune diseases. Recent findings demonstrate that IFN-γ can enhance neurogenesis in the hippocampus of adult mice, by unknown mechanisms, possibly involving coordination between brain inflammation and repair. It is also capable to modulate neurotransmitter release at synapses and affect memory, thereby revealing an important role of this immune mediator for the adult nervous system function. Using different neuroblastoma cell lines we are analyzing the influence of elements involved in the neuroinflammatory response, in the process of aberrant activation of key signaling pathways and the induction of early neuronal differentiation markers. We observed that treatment with IFNγ reduces the proliferation capacity of neuroblastoma cells. Moreover, neuronal characteristics of the cells are promoted, as indicated by the extended neurite outgrowth, the varicosity formation and the induction of neuronal differentiation markers. The data also reveal that one of the classical immune pathways that lead to MHC class II production is active in these neural crest-derived cells, and that it is progressively attenuated as the cells proceed in their differentiation program.

P101 Lentiviral-driven overexpression of miR377 in human nucleus pulposus cells induces chondrocytic phenotype genes

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Degeneration of intervertebral disc-IVD, primarily due to aggrecan degradation in the extracellular matrix-ECM of the resident nucleus pulposus cells-NPCs, is the principal cause of lower back pain in humans and has led research to understand NPC differentiation mechanisms. Recently, miRNA screenings during chondrocyte differentiation in mouse embryos revealed a dramatic increase in microRNA-377 expression. Therefore, we used a lentivirus-delivery system to overexpress hsa-miR377 in primary cultures of human NPCs and investigate its potential role in promoting expression of ECM molecules. We found that the signature chondrocytic markers aggrecan and Collagen type II in NPCs transduced with lenti-miR377 were significantly increased as compared to cells exposed to scramble miRNA, and that *sox9*, a gene responsible for the initiation of chondrogenesis was downregulated, all indicating that miR377 acts on late phenotypic genes. To delineate the signaling pathway that induces miR377, we used an array of pharmacological agents and biochemical and molecular approaches and identified PKCε as specifically promoting a chondrogenesis-like sox9 expression pattern. Next, we found that long-term activation of PKCε, through activation of ERK, upregulated the expression of AP1, CREB, miR377, and aggrecan and decreased its aggrecanase ADAMTS5. Moreover, bioinformatics analyses showed that these genes may be part of the same differentiation program, as we identified AP1 and CREB1 sites on *ACAN* promoter and clear evolutionary gains in CREB1 and AP1 sites for *hsa-miR377*. Collectively, these results define a differentiation transcriptional program in NPCs and may provide a novel tool for *in situ* therapies in the disk.

P102 Nephrin, a transmembrane protein of glomerular epithelial cells, is involved in pancreatic beta-cell survival signalling

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Nephrin, a cell surface signaling receptor, contributes to the regulation of podocyte function in health and disease. Pancreatic β-cells express nephrin; however its function in these cells remains largely unknown. To this end, we used an immortalized insulin-secreting mouse pancreatic β-cell line (βTC-6 cells), to study the potential role of nephrin in β-cell survival signaling. We demonstrated that βTC-6 cells expressed nephrin which in immunoprecipitation and co-localization studies associated with PI3K. Incubation of βTC-6 cells with functional anti-nephrin antibodies induced nephrin clustering and recruitment of PI3K to clustered nephrin at the plasma membrane. This process led to activation of PI3K, since it was associated with increased phosphorylation of Akt; this effect was inhibited by wortmannin and LY294002, indicating that Akt activation was PI3K-dependent. Pre-treatment of cells with PP1, a selective inhibitor of Src kinases, inhibited the interaction of nephrin with PI3K and Akt activation, demonstrating that Src kinases mediate the signaling downstream of nephrin. Nephrin-induced Akt activation resulted in increased phosphorylation/inhibition of pro-apoptotic Bad protein; hence nephrin–mediated PI3K-Akt activity can trigger anti-apoptotic signaling. Silencing of nephrin expression by nephrin-siRNA abolished nephrin-mediated Akt activation, suggesting a key role for nephrin in this process. Moreover high glucose impaired nephrin-mediated Akt activation without affecting nephrin expression; this effect was associated with increased nephrin endocytosis and upregulation of PKCα expression. Our findings revealed that nephrin is involved in pancreatic β-cell survival signaling and demonstrated that glucose-induced changes in nephrin signaling could lead to β-cell apoptosis in pathological conditions such as type 2 diabetes.

P103 Oxygen-glucose deprivation promotes differential expression of Grp75, Grp78 and Grp94 inflicting autophagy in a PC12 hypoxia model

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Ischemia-hypoxia is a stressful event leading to cell death and loss of neurons with implications in a variety of pathological conditions including traumatic brain injury and stroke. Previous proteomics work implicated the participation of glucose regulated proteins (GRPs) as part of the cellular response to the hypoxic stress. GRPs, known as chaperone proteins, play a central role in the correct folding of proteins under stressful conditions. Herein we present data on the expression of GRP proteins during oxygen-glucose deprivation (OGD). PC12 cells (rat pheochromocytoma) were subjected to OGD by incubation to a 2% O_2, 5% CO_2, 93% N_2 atmosphere, for 16 hours in complete medium (DMEM) depleted of sodium pyruvate and glucose. Electron microscopy on PC12 cells exposed to OGD revealed extensive vacuolization consistent with cytoplasmic degradation while histochemical staining showed acidification of the cytoplasm. Proteins were analyzed by Western Blotting and mRNA by Real Time PCR. Grp75 was decreased both at protein and mRNA level whereas Grp78 and Grp94 were upregulated. Since molecular chaperones are implicated in cellular death through autophagy different forms of LC3 were investigated. Our data showed a shift of LC3-I to LC3-II form in PC12 cells exposed to OGD. Furthermore, tunel assay was performed yielding a negative result indicating that the process of apoptosis was not activated. In conclusion, the detected increased levels of Grp78 and Grp94 may correlate with apoptosis inhibition and promotion of autophagy, while the lower expression of Grp75 might be involved in another cell protective mechanism of mitochondrial-reticulum interactions already reported.

P104

PHOSPHORYLATION OF THE M3/6 DUAL-SPECIFICITY PHOSPHATASE UPON TREATMENT WITH APOPTOSIS-PROMOTING CANCER THERAPEUTIC AGENTS

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The mitogen-activated protein kinase (MAPK) signal transduction pathways are important for the translation of environmental stimuli to the appropriate biological responses. A member of the MAPK superfamily is the c-Jun N-terminal kinase (JNK), which is activated by diverse stress stimuli and is associated with the pathogenesis of a variety of human diseases. The specific outcomes upon activation of the JNK pathway critically depend on the intensity and duration of signal transmission. M3/6 (DUSP8) is a JNK-specific dual-specificity phosphatase, which plays a very important role upon stress stimulation, by modulating the extent of JNK phosphorylation and activation. We are using two agents in our studies in order to activate the JNK signaling pathway - arsenite and phenethyl isothiocyanate (PEITC). These compounds among other cellular effects, have been shown to exhibit anticancer activity in various systems via the induction of JNK-mediated apoptosis. Interestingly, the activation of JNK by both compounds is accompanied by the phosphorylation of the M3/6 phosphatase – an effect not observed with any other MAPK phosphatase. We have used mass spectrometry to map the JNK-induced phosphorylation sites on M3/6 upon arsenite treatment, and have mutated them by site-directed mutagenesis. Our results show that phosphorylation of the M3/6 phosphatase by JNK in response to arsenite results in attenuation of phosphatase activity and acceleration of JNK activation. We are currently investigating whether the same regulation is taking place upon PEITC stimulation, and whether M3/6 phosphorylation is a common mechanism upon apoptotic JNK signalling.

P105

Pitx2+ interneuron subsets in the spinal cord: function and connectivity

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The executive component of movement- the task of determining which muscles to activate, how intensely and for how long- depends on neural circuits located in the spinal cord. At the core of these circuits are local interneurons that regulate the pattern and frequency of motor neuron firing through a combination of direct excitation, inhibition and neuromodulation. The transcription factor Pitx2 defines a set of spinal interneurons that further fractionates into V0c cholinergic and V0g glutamatergic subsets. These subsets derive from the same progenitor domain and are the smallest identified so far. These characteristics point to the possibility that they participate in the same circuit. Analysis of their connectivity performed in our lab revealed that V0c form synapses on V0g somata and proximal dendrites and vice versa providing the first evidence of their communication. V0c represents the sole source of C boutons, the first identified spinal modulatory input to motor neurons. Behavioral analysis of mice, in which the output of V0c neurons had been genetically inactivated, demonstrated impairment in a locomotor task-dependent increase in motor neuron firing and muscle activation. Because of the abundance of the C boutons on motor neurons, a more severe phenotype was expected. Furthermore, the synapse persisted after the inactivation. This led to the hypothesis that a second neurotransmitter exists. Indeed, our recent data indicate that the neurotransmitter Cart (cocaine amphetamine regulated transcript) is present in somata and terminals of V0c neurons. Confocal microscopy and the use of volocity software confirmed Cart presence in the presynaptic space.

P106 Pleiotrophin-induced signaling to endothelial cell migration involves generation of reactive oxygen species

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Pleiotrophin (PTN) is an 18 kDa secreted heparin-binding growth factor that induces cell migration through binding to both its receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) and integrin $\alpha_v\beta_3$. In the present work, we studied the effect of exogenous human recombinant PTN on the generation of reactive oxygen species (ROS) in human endothelial cells and the involvement of ROS in PTN-induced cell migration. PTN significantly increased endogenous ROS levels in a concentration and time-dependent manner. RPTPβ/ζ inhibition using genetic and pharmacological approaches or inhibition of c-src kinase activity abolished PTN-induced ROS generation. A synthetic peptide known to block PTN-$\alpha_v\beta_3$ interaction abolished PTN-induced ROS generation, suggesting that $\alpha_v\beta_3$ is also involved. The latter was confirmed in CHO cells that do not express $\alpha_v\beta_3$ or over-express wild-type β_3 or mutant β_3Y773F/Y785F. PTN increased endogenous ROS levels in cells expressing wild-type β_3, but not in cells that do not express $\alpha_v\beta_3$ or express mutant β_3Y773F/Y785F, similarly to what we have previously shown for PTN-induced migration. In the same line, in human glioma M059K cells that do not express $\alpha_v\beta_3$ and do not migrate in response to PTN, ROS levels are not affected. Inhibition of PI3K activity or Erk1/2 activation abolished PTN-induced ROS generation, suggesting that ROS production lays down-stream of PI3K and Erk1/2 activation by PTN. Finally, catalase, apocynin, AEBSF, VAS2870 and allopurinol blocked PTN-induced ROS generation and cell migration, suggesting that ROS production involves both NADPH and xanthine oxidase, and is required for PTN-induced cell migration through the cell membrane functional complex of $\alpha_v\beta_3$ and RPTPβ/ζ.

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P107 SOD-Catalase dipole activity as a marker of a well-balanced antioxidant response in endothelial cells

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An imbalance between pro-oxidants and antioxidant systems is implicated in the physiopathology of endothelial dysfunction. The action of pro-inflammatory factors in the vasculature has been studied extensively, but the balance between endothelial antioxidant mechanisms and its relation to oxidative stress is not well established. Thus, the aim of the present study was to investigate the effect of known acute pro-oxidant stimuli in SOD and Catalase activity balance and ROS (reactive oxygen species) removal in macroendothelial cells.

EAhy926 cells were exposed to 20ng/ml TNF-α or 25.5mM Glucose for 2 hours, or were co-cultured with macrophagic cell population, which was left naïve or was further stimulated by LPS (lipopolysaccharyde). SOD and Catalase activities were estimated in total cell lysates and intracellular H_2O_2 levels by *fluorescence*.

At the above pro-oxidant experimental models, intracellular H_2O_2 and total SOD and Catalase activity increased significantly ($p<0.001$), apart from 25.5mM Glucose, which kept both enzymes activity lower than control levels ($p<0.001$). Interestingly, all stress factors caused a significant ($p<0.001$) SOD-Catalase activity imbalance, where SOD activity was higher ($p<0.001$) than Catalase activity compared to untreated cells.

According to the results, TNF-α, hyperglycemia and macrophagic populations increase the intracellular ROS burden, while the antioxidant response of SOD and Catalase is also stimulated, except of hyperglycemic conditions. SOD possesses a prominent role versus Catalase, which may result in the observed H_2O_2 accumulation. Conclusively, the short-term effect of endogenous and exogenous pro-oxidant/inflammatory factors on endothelium is dependent on the well-balanced first-line response of SOD-Catalase enzymic dipole to ROS neutralizing process.

P108

Specific amino acids in hepatitis C virus (HCV) core proteins derived from genotypes 1 and 4 are responsible for the upregulation of Wnt/β-catenin downstream genes, Tbx3, c-myc and cyclin D1

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The multifunctional HCV core protein is implicated in the development of hepatocellular carcinoma (HCC) caused by HCV infection, but the underlying mechanism is not fully understood. Activation of the Wnt/ β-catenin pathway plays a major role in HCC and is modulated by the HCV core protein. HCV is characterized by extensive genetic diversity and different clinical isolates do vary in their infectivity and pathogenesis. The aim of this study is to determine the possible influence of genetic variability in HCV core protein in enhancing the Wnt/ β-catenin signaling activity and to elucidate the molecular mechanisms by which HCV core modulates activation of β-catenin. The Wnt/β-catenin activity was investigated in transiently transfected HEK 293T and Huh 7.5 cell lines transiently expressing HCV core proteins from HCV genotypes 1a, 4a, 4f and from a unique isolate of genotype 1a obtained from a Cambodian patient (1aCam). Luciferase-based reporter assay, Western blot, qPCR, mutagenesis and immunofluorescence methods, were used to measure gene and protein expression levels. We found that, HCV core protein upregulates β-catenin-mediated Tcf-dependent luciferase activity in a genotype specific manner. Consistent to these findings, HCV core stabilizes β-catenin levels, induces nuclear translocation of the protein and inactivate GSK3β activity. Moreover, specific amino acids in HCV core sequence found to be responsible for Tcf-element transactivation. Finally, we showed that HCV core upregulates the expression of Tbx3, c-myc and cyclin D1 genes. In conclusion, HCV core protein from different genotypes appears to differentially regulate the Wnt/β-catenin signaling pathway and this finding may contribute to different potential of HCV genotypes to induce HCC.

P109

Synergism of TGF-β1 with MIF in the production of IL-6 by human pterygium fibroblasts

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Pterygium is a condition characterized by epithelial overgrowth of the cornea, inflammatory cell infiltration and an abnormal extracellular matrix accumulation. Chronic UV exposure is considered as a pathogenetic factor of this disease. IL-6 is a multifunctional cytokine implicating in a variety of inflammatory conditions. TGF-β1 has been traditionally considered as a predominantly immunosuppressive and anti-inflammatory mediator. Both IL-6 and TGF-β1 play a significant role in enhancing cell proliferation and extracellular matrix formation. MIF is a predominantly pro-inflammatory cytokine that plays a key role in several inflammatory and autoimmune diseases. The aim of this study was to investigate the implication of these factors in pterygium pathogenesis.

RT-PCR analysis showed that the expression of MIF as well as of IL-6 was higher in pterygium than in normal conjunctiva, while no difference was observed in the expression of TGF-β1 between of two tissues. Upon UVB exposure of pterygium fibroblasts at different radiation doses, a dose-dependent stimulation of MIF and IL-6 production was observed. Both TGF-β1 and MIF were able to enhance the expression of IL-6 in pterygium fibroblasts in a dose-depended mode. When the two factors were used together, a synergistic effect in production of IL-6 was observed.

In conclusion, it appears that the UVB radiation leads to stimulation of production by cells in eyes conjunctiva of factors which are implicated in extracellular matrix accumulation and consequently they may contribute in pterygium formation.

P110 The PP4R1 subunit of protein phosphatase PP4 negatively regulates NF-κB activation by modulating the phosphorylation of TRAF2

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TRAF2 is an E3-ubiquitin ligase that is implicated in the activation of MAPK and NF-kappaB pathways by members of the TNF receptor superfamily. In order to identify regulators of TRAF2 activity, a yeast two-hybrid screen was performed using the amino terminal 358 amino acids of TRAF2 as bait. One of the proteins that were identified by this screen was the PP4R1 regulatory subunit of protein phosphatase PP4. The interaction of PP4R1with TRAF2 was verified by the coimmunoprecipitation of the two proteins and it was shown to be dependent on the RING finger of TRAF2, which is essential for the ubiquitin ligase activity of TRAF2. Overexpression of PP4R1 inhibited TRAF2-mediated activation of NF-kappaB. Phosphorylation of TRAF2 at serine 11 was previously shown to be important for JNK and NF-kappaB activation by TNF. Therefore, the possible role of PP4R1 in the modulation of TRAF2 phosphorylation at serine 11 and function was investigated in epithelial cells. Exogenous expression of PP4R1 reduced TRAF2 phosphorylation at serine 11. Furthermore, coexpression of the catalytic subunit of protein phosphatase PP4 (PP4c) but not its inactive mutant, enhanced the dephopshorylation of TRAF2 serine 11 that is mediated by PP4R1 overexpression. Downregulation of PP4R1 by RNA interference enhanced the phosphorylation of TRAF2 at serine 11. These results identify the catalytic subunit of protein phosphatase PP4 and its regulatory subunit PP4R1 as modulators of TRAF2 phosphorylation and function as a mediator of NF-kappaB activation by TNF.

P111 The role of Arf6 in ActivinA/TGFβ signaling

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ARF6 is a small molecular weight GTPase localizing to the plasma membrane and endosomal compartments where it regulates endocytic membrane trafficking and actin remodeling. As ARF6 cycles through its active and inactive conformations it regulates cell surface ligand internalization, post-internalization trafficking along the endocytic pathway, and endosomal recycling and fusion of an endosomal membrane with the plasma membrane. Through it's effector proteins Arf6 affects many cellular functions including cell motility, adhesion, abscission and lipid homeostasis.

Here we describe a novel role of Arf6 in regulating ActivinA/TGFb responses. The ActivinA/TGFβ family ligands signal through heteromeric complexes of type I and type II transmembrane serine/threonine kinase receptors, which phosphorylate SMAD2/3 proteins. The phosphorylated SMAD2/3 proteins oligomerize with SMAD4, translocate to the nucleus and regulate transcription using a large network of interactions with transcription factors, co-activators and co-repressors.

A yeast-two-hybrid approach initially indicated a potential interaction between Arf6 and SMAD4 and further analysis validated this result. Mapping of the interacting region showed a direct interaction of Arf6 with the C-terminus tail of SMAD4 and also with the highly similar tails of SMAD2 and SMAD3. We have addressed the significance and regulation of these results and present our findings.

P112

The role of heparin and its nano-modified forms in proteasome activity regulation and functional properties in breast cancer

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Heparin and its derivatives may exhibit anti-cancer activity, except from their anticoagulant action. Therapeutic approaches are based on the administration of unfractionated heparin and low molecular weight heparin, of longer half-life, as well. 26S Proteasome is a protein complex that consists of catalytic and regulatory subunits, involved in protein degradation. The β1, β2, and β5 subunits are catalytic; although they share a common mechanism, they have distinct substrate specificities considered chymotrypsin-like, trypsin-like and caspase-like. Modifications of its action may be a key in targeted cancer therapy. We have investigated the effects on cell functional properties (proliferation & motility), as well as on proteasome activity of LMWH, UFH and two heparin nano-derivatives, one extracted from a marine invertebrate Ascidia and a second one porcine intestinal heparin, in two breast cancer cell lines (MDA-MB-231 & MCF-7). A dose-dependent effect was observed on cell viability as regards all heparins, while cell migration was induced after treatment with nano-heparins and heparins, with the exception of porcine intestinal heparin where an inhibitory effect was observed in MCF-7, whereas in MDA-MB-231 this effect was present only in low concentrations. Furthermore, RT-PCR analysis of proteasomal catalytic subunits on MCF-7 and MDA-MB-231 breast cancer cells showed an upregulation and a slight downregulation, respectively, both treated with different types of heparin. However, proteasome activity levels were not affected with all heparin derivatives, with an exception in the case of heparin extracted from Ascidia, which significantly reduced proteasome activity in MDA-MB-231 cells, while in MCF-7 proteasome activity presented upregulated. Conclusively, it is of great importance that heparin and its nano-derivatives have the ability to regulate breast cancer cells' functions and proteasomal activity at certain concentrations. These primary results indicate that heparin and its nano-forms except from anticoagulant activity may have a potential anticancer action in breast cancer cells.

P113

Time-dependent expression of Hsc70, Grp75, Grp78 and Grp 94 in a Rat pheochromocytoma (PC12 cells) excitotoxicity model

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Glutamate is the major excitatory neurotransmitter in the central nervous system. Excessive glutamate becomes toxic through overactivation of glutamate receptors and this situation is called excitotoxicity. Excitotoxicity leads to intracellular accumulation of Ca^{2+}, activation of molecular pathways related to intracellular stress and the expression of proteins which exhibit chaperone activity. These are Hsc 70 and the GRP family of proteins (glucose-regulated proteins), such as Grp 75, Grp 78 and Grp 94. Grps mediate neuroprotection by preventing protein misfolding, oxidative stress and upregulation of nitric oxide synthase. Grps also suppress the activation of the stress-activated protein kinase JNK, which is related to stress apoptotic pathway. In addition, Hsp70 is essential for ubiquitin-dependent protein degradation.

In this work we investigated the expression of the above mentioned Grps and Hsc 70 in a rat pheochromocytoma PC12 excitotoxicity model. PC12 cells were incubated with different concentrations of glutamate for various time intervals. A functional excitotoxicity model was established at 10μM glutamate for up to a maximum of three hours. In particular, exposure of PC12 to 10μM glutamate for 30min, 1h, 2h and 3h provoked a time-dependent upregulation of Grps and Hsc 70 expression, peaked at 2h of exposure, after which a decrease was observed comparable to their normal levels. Furthermore, histochemical staining revealed swollen cells with limited cytoplasm compared to control, an observation verified by electron microscopy that showed disorganization and vacuolization of the endoplasmic reticulum leading to vesicular cytoplasmic degeneration. In conclusion, our data propose a cytoprotective role of chaperones under stressful conditions.

P114

Twin presequences in plants: evidence for ACP1 dual-targeting to mitochondria and chloroplasts

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Dual-targeting of proteins to mitochondria and chloroplasts has been surprisingly frequent due to their post-endosymbiotic evolution. Two types of dual-targeting presequences have been suggested in plants. The ambiguous presequence generating a single protein isoform with a targeting peptide domain recognized by the import apparatus of both mitochondria and chloroplasts. The twin presequences including two separate targeting domains arranged in tandem at the N-terminus initiated from two in-frame AUGs. Bioinformatics analysis of the Arabidopsis genome identified a small number of candidate genes with twin presequences for dual-organellar targeting. The acyl carrier protein1 (At3g05020) was identified with a twin presequence configuration involved in the process of fatty acid biosynthesis. The N-terminal extension bears 63 amino acid residues corresponding to a putative mitochondrial presequence followed by the chloroplast transit peptide. To experimentally test the dual-localization pattern, the *YFP* reporter gene was fused in-frame at the 3' end of *ACP1* gene. The expression of these constructs was driven by the constitutively expressed *CaMV35S* promoter and various cell types of transient and stable transformants were analysed *in vivo* by fluorescence microscopy. Cell imaging analysis of the *ACP1-YFP* construct, containing the two in-frame AUG codons, revealed simultaneous ACP1 targeting to chloroplasts and mitochondria both in the homologous (Arabidopsis) and heterologous (*Nicotiana benthamiana*) system. Given that lipid biosynthesis in plants is mainly performed in the stroma of chloroplasts, these results provide evidence of a dual compartmentalized biochemical pathway additionally in mitochondria most likely as a legacy of their endosymbiotic heritage.

P115 Identification and characterization of natural and synthetic compounds potentially effective in preventing osteoporosis

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Postmenopausal osteoporosis is a major cause of morbidity among the elderly, women in particular. The current treatment regimes include among others hormone (estrogen) replacement therapy (HRT) and selective estrogen receptor modulators (SERMs) such as raloxifene. Nonetheless, exogenous estrogen has been shown to increase breast and uterine cancer risk and raloxifene to be more safe but less effective in preventing osteoporosis compared to HRT. Plant derived supplements are used by numerous women to prevent and/or treat postmenopausal degenerative diseases. Aim of our study is to identify and characterize plant derived compounds and derivatives thereof that display functional and structural similarity to estrogen (i.e. phyroestrogens) and/or are capable of safely preventing postmenopausal osteoporosis. The major objectives of our study include: i) screening a large compound library using as readout, a)differentiation of RAW264.7 cells to osteoclasts and MC3T3-E1 cells to osteoblasts, b) cell–based estrogenic responses and c), cell–based agonist/antagonist responses through estrogen receptor alpha and beta, ii) testing breast cancer risk of selected compounds using inhibition of mammary epithelial cell differentiation as readout, iii) analyzing the mechanism of action of selected leads based on their transcriptomic profiles on RAW264.7 and MC3T3-E1 cells and finally, iv) evaluating the osteoprotective effect of selected leads using animal models of ovariectomy-induced post-menopausal osteoporosis. Here, we present the evaluation of *in vitro* osteoprotective, estrogenic and ERα and ERβ agonist/antagonist activity of 200 natural and synthetic compounds their effect on mammary epithelial cell differentiation using estradiol and raloxifene as positive controls. In addition, we examine the relationship between their *in vitro* osteoprotective and/or estrogenic performance and their role as ERα and/or ERβ agonists.

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P116 RpS5, from the nucleus to the cytoplasm

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Ribosomal protein S5 is mainly localized within the nucleolus, but is also found in the cytoplasm. Our research team has been studied, in a way, the transport of rpS5 from the cytoplasm to the nucleus and then to the nucleoli. It seems that rpS5 enters the nucleus with the flexible region 38-50aa serving as a structural element. Upon its entry to the nucleus, the C-terminal and N-terminal cooperate, (Thr-133 undergoes phosphorylation by kinase CKII, which triggers Ser-24 phosphorylation promoting protein's accumulation into the nucleoli).

Therefore, we thought that it would be interesting to examine the way that rpS5 leaves the nucleus for the cytoplasm, (the reverse way), and its possible contribution to the export of "semi-mature" 40S subunit. We made constructs of rpS5 fused with GFP, and we have studied its subcellular localization, with the presence of leptomycin B, a chemical inhibitor of the nuclear transport receptor Crm1. Leptomycin B disrupts the ternary complex, causing nuclear accumulation of rpS5 to nucleus.

GFP-rpS5-Thr8 and GFP-rpS5-Thr14 mutant resulted to the accumulation of S5 protein into the cytoplasm, suggesting that mutated S5 was unable to enter the nucleus. On the other hand, the GFP-rpS5-Ser-125 mutant seems to be essential for entering the nucleoli. These results corroborate ours hypothesis about a possible cooperation between C-terminal and N-terminal region for entering S5 to cytoplasm.

P117

A Time-Course study of Lead-induced effects on Acetylcholinesterase activity in adult female mouse brain

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Lead (Pb), a neurotoxic bio-accumulative heavy metal, is associated with cognitive dysfunctions. Our previous studies showed that lead administration to adult mice affected acetylcholinesterase (AChE) activity, an enzyme which is involved in acetylcholine metabolism, in a tissue-specific and dose-dependent way (250 ppm/500ppm). The aim of the present study was to investigate whether a time-dependent Pb administration (15-30-60 days) affected brain acetylcholinesterase (AChE) activity of specific brain regions (cerebral cortex, cerebellum, midbrain, striatum, hippocampus and diencephalon) in female adult mice. Animals were randomly divided into 6 groups. Mice of the first 3 groups were given orally 250 ppm $Pb(CH_3CO_2)_2$ dissolved in drinking water for 15,30,60 days respectively. Respective control animal groups were also prepared. On the completion of each Pb treatment the animals were sacrificed. AChE activity was determined in both salt-soluble (SS-AChE) and detergent-soluble (DS-AChE) fractions of all brain regions tested, by Ellman's colorimetric method. Our results showed that Pb administration decreased both SS- and DS-AChE activity in all the brain regions of each group. Moreover, the decrease was noted to be proportional to the period of Pb administration with diverse tissue-specific changes. Conclusively, our results show that the time-dependent decrease of AChE activity, in Pb-treated animals, simulates to the dose-dependent Pb administration effects on AChE activity, obviously because of the Pb accumulation in brain.

P118

Activity, anti-cancer effect and nanodelivery of new anilinoquinazoline EGFR inhibitors

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Epidermal Growth Factor Receptor (EGFR) is a ubiquitous tyrosine kinase that modulates cell physiology (1). Binding of physiological ligands, such as EGF, to EGFR alter cell differentiation, proliferation and migration and control cell survival. In a number of malignancies, including those of the lung, breast, colon, and kidney, EGFR is overexpressed or hyperactivated leading to aberrant cell signaling (2). Targeting EGFR with tyrosine kinase inhibitors (TKIs) constitutes an important line of defense against EGFR-positive cancers, however, inevitably, resistance develops. Recently discovered second generation TKIs, like the drugs gefitinib and lapatinib, are effective against some resistant tumors (3). Chemically, they both comprise an anilinoquinazoline scaffold that specifically recognize the ATP-binding pocket of the cytoplasmic, kinase domain of EGFR.

We aim to develop new anilinoquinazoline EGFR inhibitors and use them to direct nanodevices to cancer cells and EGFR-resistant tumors for both targeted therapy and imaging (theranostics). A dozen of new type-I anilinoquinazoline derivatives have been synthesized. We show their EGFR kinase inhibitory activity *in vitro* and how they affect cell survival of HeLa and MCF-7 cells, untreated or after stimulation with EGF. Using confocal microscopy, we present preliminary imaging data that demonstrate the interaction of promising fluorescently labeled nanosystems, with living cells. We finally illustrate Surface Plasmon Resonance (SPR)-based approaches to quickly screen the functional loading of the nanosystems in grafted anilinoquinazolines. The potential of using these results for the construction of efficient, EGFR-specific, cancer theranostic applications is discussed.

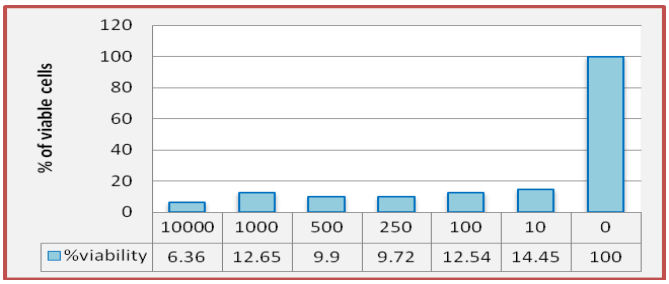

Figure 1. Viability of EGF stimulated HeLa cells as a function of increasing concentrations (nM) of a new anilinoquinazoline derivative

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P119

Development of recombinant hepcidin and specific mAbs for diagnosis and therapeutic applications of iron-homeostasis disorders

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Hepcidin is a 25-aminoacid cysteine-rich iron regulating peptide. Increased hepcidin concentrations lead to iron sequestration in macrophages, contributing to the pathogenesis of anaemia of chronic disease. Conversely, decreased hepcidin is observed in iron deficiency and iron overload diseases, like hereditary haemochromatosis. Because of its profound biomedical significance, hepcidin has become the target of intense biochemical studies. Due to its structure, the production of the peptide itself, as well as monoclonal antibodies (mAbs) against it, is particularly difficult. In previous studies, we have reported the expression, purification and functional characterisation of a 6-His-tagged hepcidin variant in yeast *P. pastoris*. In this study we report:

a) the characterisation of mAbs produced against native hepcidin, where different immunogens containing hepcidin were used. We produced several specific monoclonal antibodies that immunoprecipitated native hepcidin peptide overexpressed in supernatants of Huh7 cells stably transfected with the hepcidin cDNA,

b) the purification of monomeric untagged Hep-25 peptide expressed in the supernatant of *P. pastoris, which* was achieved by using these mAbs in affinity chromatography, with a yield of 0.5 mg/l of culture. This peptide was capable of internalising ferroportin-gfp protein from the surface of Hek293 cells,

c) an ELISA assay that was developed for measuring hepcidin serum concentration with the use of recombinant Hep-25 peptide and a monoclonal antibody. This assay may replace the previous one that has been developed by utilising a polyclonal antibody and the Hep25-His variant.

The potential treatment of anaemia of inflammation by using these mAbs is currently under investigation.

P120

DNA BINDING AND BIOLOGICAL STUDIES OF TWO NOVEL Ga (III) PORPHYRINS

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In recent years, a great deal of studies has been focused on the use of metal complexes as chemotherapeutic agents and DNA probes [1-3]. In addition, the unique ability of cationic porphyrins to associate with nucleic acids, especially DNA, has led to studies of medical and biological applications of these kind of ligands [4]. As a result more and more therapeutic applications such as photosensitizers in photodynamic therapy, inhibitors of DNA and anticancer agents are increasingly gaining ground. Porphyrins as photosensitizers can localise the tumor cells, produce the singlet oxygen, cleave with DNA and finally destroy selectivity the tumor cells.

In this study, we present the DNA affinity of two novel gallium (III) porphyrins, the TPyP (meso-tetrakis pyridiumyl porphyrinato) Ga (III) and the water- solube TEPyP (meso-tetrakis ethyl pyridiumyl porphyrinato) Ga (III) [5] , which both are derived from meso-tetrakis (N-methyl-4 pyridumyl) porphyrin (H_2TPyP). Their interaction with calf thymus DNA was investigated by a wide range of techniques including UV-Vis, circular dichroism, and agoraze gel electrophoresis. The reference compounds were also evaluated for their potency to inhibit the tumor cells growth by the MTT assay[6]. Taking into consideration all the data of the aforementioned measurements the interaction of both complexes with DNA has been established. In any case the photocleaving ability has been proven, via a mechanism involving singlet oxygen. In the light of these results, it is obvious that these porphyrins could be the potent sensitizers in photodynamic therapy (PDT) in the near future.

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P121 MDR-involved human glutathione transferases (hGSTs) are targets for inhibition by 2,2'-dihydroxybenzophenones and N-carbonyl analogues

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GSTs are a family of medically important isoenzymes that catalyse the conjugation of glutathione (GSH) to a variety of hydrophobic xenobiotic compounds, rendering them hydrophilicity and facilitating their metabolic processing and secretion. Based on the same detoxification mechanism, cancer cells may acquire resistance by overexpressing GST activities, thus, hampering the effectiveness of certain chemotherapeutic drugs. Therefore, several drugs and prodrugs, acting as inhibitors against human GSTs, have been proposed to overcome multiple drug resistance (MDR) attributed to GST overexpression. On the other hand, benzophenones constitute a major class of compounds found in plants, exhibiting cytotoxic activity in several cancer cell lines, as well as antimicrobial, antiviral and antioxidant activities. Substituted 2-hydroxybenzophenones are ubiquitous in naturally occurring and synthetic compounds exhibiting important biological activities. Our recently reported studies on xanthone synthesis as well as its inhibitory potential towards human GSTs (hGSTs), prompted us to scrutinize its ring-opened form, substituted 2,2'-dihydroxybenzophenones and N-carbonyl analogues, taking advantage of their structure similarities, in pursuit of identifying new lead compounds as inhibitors against hGSTs involved in MDR. Following GST inhibition screening, *in silico* molecular docking and GST inhibition kinetics, we identified analogues with strong inhibitory potency (IC_{50} values from 0.18 to 1.8 μM) and low cytotoxic activity for Caco2 cell line (LC_{50} values from 35 to > 400 μM) useful as lead structures in designing new inhibitors and respective prodrugs for human GSTs.

P122 The flavour compounds of traditional Greek cheeses and their variants: a review of current knowledge

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This work presents a review of the flavour compounds of traditional Greek cheeses, such as Feta, Kopanisti, Kefalograviera and their variants. Cheese flavour originates from the indigenous milk enzymes, the added rennet and cultures and the various processes that take place during cheese making and storage: primarily the catabolism of lactose, protein, lipid, citrate and lactate. Alcohols were the most abundant group of flavour compounds of feta cheese, with ethanol, 3-methylbutanol and pentanol the major alcohols in all cheeses at 60 days of storage. Butan-1-ol, heptan-2-ol and phenylethanol contents were significantly higher in feta cheese ripened and stored in wooden barrels than that in tin vessels at both sampling times. Esters were the third most abundant group of flavour compounds and occurred mainly in the form of ethyl esters. Feta cheese ripened and stored in wooden barrels contained larger quantities of ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl lactate, ethyl decanoate, 3-methylbutyl acetate and phenylethyl acetate than Feta cheese ripened and stored in tin vessels at both sampling times. The main volatile compounds detected in Kopanisti cheese included ethanol, 2-butanol, 2-butanone, pentanal and several ethyl esters. Alcohols accounted for 60% of the total volatile compounds owing to the high concentration of ethanol found in the samples. Esters were the second most significant group of aromatic compounds in Kopanisti after alcohols, representing 26% of the total aromatic compounds. The principal flavour compounds found in Kefalograviera cheese were ethanol, acetoin, 2-butanol, 2-butanone and acetaldehyde.

P123

The provoked cytotoxicity of airborne particulate matter (PM) of small size on human lung cell line MRC-5

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Taking into consideration the generated adverse effects after the exposure to urban airborne particulate matter (PM), we investigated the biological impact of size segregated PM (<0.49, 0.49-0.97, 0.97-1.5, 1.5-3, 3-7.2 and >7 μm) collected in Thessaloniki, during the winter season of 2012-2013. In particular, the capacity of the aforementioned particle fractions to induce cytotoxic responses in human lung cells (MRC-5) in cultures was studied both by measuring the activity of cellular mitochondrial dehydrogenase (MTT test) and the activity of cytoplasmic lactate dehydrogenase (LDH). The two tests were applied on aqueous extracts of the various particle size fractions and on organic solvent extracts containing non-polar, medium- polar and polar organic contaminants.

MTT assays showed that, after 48 h of exposure on MRC-5 cell line, the polar organic extracts of the finest PM fraction (<0.49 μm) from the traffic site, are the most cytotoxic. On the contrary, further subjection of the more toxic aforementioned PM fractions under LDH investigations showed that although their measured cytoplasmic LDH activity was negligible, the observed significant cell morphological changes suggest apoptotic mechanisms which are corroborated by chromosomal DNA fragmentation of MRC5 cells.

P124

Saffron administration protects from selenite-induced cataract via calpain inhibition

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We have previously demonstrated the anti-cataractogenic potential of saffron (*Crocus sativus* style extract rich in crocins) via reinforcement of lens antioxidant defense. The purpose of this study was to examine the effects of a different saffron administration scheme on lens calpains, calcium-depended cysteine proteases with a crucial role to cataractogenesis. New born suckling Wistar pups were randomly divided in three groups: "Se" (20 μmol Na_2SeO_3/kg subcutaneously – post natal (PN) day 10), "Se+Saffron" (20 μmol Na_2SeO_3/kg subcutaneously – PN day 10/60 mg dry weight of saffron methanolic extract/kg intraperitoneal – PN day 11 & 12), "control" (saline on the respective days). On PN day 21, clinical examination revealed that 62.5% of the lenses of "Se+Saffron" group were clear, showing that saffron administration protected rat pup lenses from cataract formation; "Se" median:2 (range 2-3), "Se+Saffron" median: 0 (range0-1), $p<0,001$. The fluorometric determination of total lens calpain activity showed a significant reduction (44.15%,$p<0.01$) in the "Se+Saffron" group in comparison to the "Se". The small but statistically significant increase (14.45%) of the ratio of lens soluble to insoluble proteins in the "Se+Saffron" group was further supported by SDS-PAGE electrophoresis results, where the degradation of proteins of ~10-30 kDa in the "Se" group was not observed when saffron was co-administered. In order to elucidate the mechanism of inhibition, we investigated *in vitro* the effect of trans-crocetin, the main crocin metabolite, on μ-calpain. Trans-crocetin exhibited strong inhibitory activity; IC50 of 145 nM. Conclusively, saffron anticataractogenic potential is also attributed to the prevention of protein degradation through calpain inhibition via its metabolite.

P125

Apolipoprotein E3 - containing HDL treatment induces alterations in endothelial cell proliferation pathways

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Introduction: Endothelial cell (EC) proliferation contributes to re-endothelialization after vascular injury, which is an initiating event in atherogenesis. Apolipoprotein E (ApoE) mediates clearance of triglyceride-rich lipoproteins and cholesterol efflux from macrophages.
Aim: To investigate the effect of reconstituted HDL containing human wild-type apoE3 and phospholipids (rHDL-apoE3) on EC proliferation.
Methods: Primary normal human aortic ECs were treated with rHDL-apoE3 or PBS (5 replicates/group). Total RNA was analyzed by whole genome microarrays (Gene 1.0 ST Array/Affymetrix), followed by bioinformatical analysis (2-fold and ≤ 0.05 FDR thresholds) and qRT-PCR validation of 23 EC proliferation-related genes. Data-mining involved cutting-edge software and extensive PubMed-based searches.
Results: Significant changes were observed in 198 genes, and 23 of these were directly associated with EC proliferation pathways: a) rHDL-apoE3 increased the expression of EGR1, ID1, PTGS2 and PDGFD, which activate the ERK1/2 pathway downstream of TIE2, VEGFR2 and FGFR1. Activation of ERK1/2 pathway promotes cell-cycle progression. The activation of ERK1/2 kinases downstream of FGFR1 is further supported by PTX3 downregulation. b) Combined rHDL-apoE3-mediated upregulation of PIK3CG and TGFB2 downregulation could activate the PI3K/AKT pathway downstream of CXCR4 and FGFRs. Activation of PI3K/AKT kinases stimulates cell-cycle G1/S-transition. c) Nine of the 23 EC proliferation-related genes (e.g.↑ID1, ↑EGR1, ↓TGFB2) directly regulate cell-cycle regulatory molecules involved in G1/S (p21/p16/p27/CCND1/CCNE1/RB) and G2/M checkpoints (p21/p57/CCNB1).
Conclusion: ***rHDL-apoE3 induces alterations at transcriptional level in EC proliferation-related pathways and in upstream regulators of molecules implicated in cell-cycle control. Through these changes rHDL-apoE3 could preserve the integrity of the endothelium and thus protect from atherosclerosis.***

P126

Secretome analysis from normal and malignant cervical cell lines employing proteomic approaches

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Cervical cancer represents the second most common malignancy in women worldwide, and is attributed to infection by high risk HPV types 16 and 18. The ensuing persistent viral infection, combined with a constitutive expression of viral oncoproteins E6 and E7, is considered a highly inductive step towards the malignant transformation. However, the eventual steps leading to cancer have not been yet elucidated. The cell secretome represents the collection of the entire macromolecules secreted by a cell, and constitutes a vital aspect of cell-cell communication. During carcinogenesis, cancer cells display secretomes with specific altered composition, reflecting the acquisition of hallmarks of cancer and probably contribute to the individual stages of carcinogenesis. In the present study, we focused for the first time on the systematic evaluation of the secretome of four cell lines, to delineate putative biomarkers and reveal pharmaceutical targets. The secretome of normal human keratinocytes HCK1T, was compared to different cervical cancer cell lines [C33-A (HPV negative), SiHa (HPV16+), HeLa (HPV18+)]. The secretome profile of SiHa and HeLa cells was analyzed by 2D-gel electrophoresis and was significantly different from the proteomic profile of the corresponding total cell extracts. Secreted proteins from the SiHa cell line were identified by mass spectrometry. Cathepsin D, a secreted lysosomal protein was upregulated in SiHa cell line compared to control HCK1T. This proteomic result was further validated by immunohistochemistry. Thus, these data identify for the first time the secretome profile of cervical cancer and provide putative biomarkers, such as Cathepsin D for further clinical validation.

P127 Comparison of drug metabolizing arylamine N-acetyltransferases (NATs) in humans and primate models

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Human arylamine N-acetyltransferases, NAT1 and NAT2, catalyze the acetyl-coenzyme A dependent N-acetylation of arylamines and arylhydrazines. NAT enzymes are involved in drug metabolism and chemical carcinogenesis. Human NAT proteins are difficult to express in *E. coli* to high levels of yield and purity and this complicates functional studies, as well as chemical screens for inhibitors. To overcome these problems, we have undertaken cloning, recombinant expression and comparative enzymatic analysis of the NAT1 and NAT2 homologues of 11 primate species, including human. Overall, the recombinant NAT1 and NAT2 proteins of non-human primates were recovered in higher levels of yield and purity (following affinity chromatography), compared with their human counterparts. Enzyme activity assays of recombinant proteins were performed with established NAT1 and NAT2 substrates using a standard colorimetric method. Highest activity levels were demonstrated for *Macaca mulatta/Macaca sylvanus* NAT1 (identical in the two species) and *Erytrocebus patas* NAT2. Differential scanning fluorimetry was used to assess the thermal stability of the expressed recombinant proteins, providing highest melting temperatures for *Nomascus gabriellae* NAT1 and *M. sylvanus* NAT2. In conclusion, our comparative studies demonstrate that the superior yield, purity and stability of phylogenetically conserved NAT homologues from non-human primates make them ideal models for *in vitro* functional, biochemical and pharmacological studies of the human NAT enzymes.

P128 Pharmacogenomic studies in the hippocampus of a mouse model for Mesio-Temporal Lobe Epilepsy

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Introduction: The MTLE syndrome is the most common form of intractable epilepsies, characterized by the recurrence of focal seizures occurring in mesio-temporal limbic structures and often associated with hippocampal sclerosis and drug resistance.
Aim: To characterize the pathogenetic mechanisms involved in epileptogenesis of MTLE in order to identify novel potential therapeutic targets.
Methods: The mouse model for MTLE was obtained by intrahippocampal microinjection of kainate (KA;1 nmol/50 nL) and compared to saline injected wild type animals. RNA was extracted from dissected hippocampi 12 hours post injection, and analyzed by Affymetrix whole Mouse Genome 430 2.0 Arrays. Significant gene expression changes were identified using the Significance Analysis of Microarrays software (fold change≥2, FDR<0.05), while the transcription factors likely orchestrating the observed changes were predicted by the ExPlain™ software.
Results: Significant changes were observed in 929 probe sets, related to proliferation and survival (Fosb↑, Junb↑), negative regulation of apoptosis (Cflar↑, Bcl2a1↑), synaptic plasticity (Homer1↑, Arc↑) including glutamate signaling (Gria3↓, Grm5↓), inflammatory response (Il6↑, Ccl2↑, Icam1↑), astrocyte (Vim↑, Serpina3n↑) and microglia activation (Cd68↑, Lgals3↑), and stress response mechanisms (Hmox1↑, Hspa1a↑). Many of the genes involved in these functional categories are predicted to be regulated by transcription factors overexpressed themselves (e.g. Fosb↑, Junb↑, Nr4a2↑).
Conclusion: During MTLE epileptogenesis synaptic plasticity is highly modulated, and a range of stress and inflammatory processes including reactive astrocytes and microglia are activated, in parallel to cell survival mechanisms. These mechanisms emerge as moderators of the neurotoxic effects, in the early stages of epileptogenesis.

P129 Polymorphic acetylation of drugs in the model primate Rhesus macaque: the role of NAT enzymes

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Arylamine N-acetyltransferases are polymorphic drug metabolising enzymes recognised as landmarks in establishing the discipline of pharmacogenetics. Humans have two functional *NAT* genes (*NAT1* and *NAT2*) and polymorphisms in both loci have been known to modulate susceptibility to the toxic effects of drugs and environmental carcinogens. We have investigated the possible presence of genetic polymorphisms in the *NAT1* and *NAT2* homologues of the Rhesus macaque, a primate species used as model in biomedical research. Screening of 25 Rhesus macaque individuals identified 12 distinct haplotypes at the *NAT1* and 12 at the *NAT2* locus. Combined, these haplotypes bear a total of 5 synonymous and 7 non-synonymous SNPs within the *NAT1* coding region, as well as 6 synonymous and 7 non-synonymous SNPs within the *NAT2* coding region. One non-synonymous polymorphism (p.Arg187Gln) is also responsible for the slow acetylator phenotype conferred by human *NAT1*14* allele. Cloning and recombinant expression of this allele demonstrated an effect on NAT1 enzyme activity similar in the rhesus and human. Certain NAT2 variants of Rhesus were also predicted by *in silico* means to potentially affect the function of the isoenzyme. These polymorphic variants were thus expressed in recombinant form for enzymatic analysis against different substrates. Our results indicate various effects on enzyme activity and/or substrate selectivity. Polymorphism p.Met42Val lowers enzymatic activity, while p.His186Asn appears to affect enzyme activity in a substrate-dependent manner. Nonsense mutation p.Ser221stop generates a non-functional protein, while p.Asp142Tyr has no phenotypic effect. Functional analysis of the remaining SNPs is currently in progress.

P130 Proteome changes of Lactococcus lactis MG1363 during exposure to the bacteriocin peptide macedocin produced by Streptococcus macedonicus ACA-DC 198

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Bacteriocins are antimicrobial peptides produced by different types of organisms in an attempt to prevail in the biological warfare taking place in their ecological niche. *Streptococcus macedonicus* produces macedocin, a lantibiotic belonging to the lacticin 481 group, whose bactericidal mode of action has rarely been investigated. *Lactococcus lactis* MG1363 which is considered as the model organism for lactic acid bacteria is sensitive to macedocin. In order to explore the killing mechanism of macedocin, we assessed changes at the proteome level in *L. lactis* mid-log phase cells when exposed to a sublethal concentration of the peptide. Differential proteomics were performed using two dimensional gel electrophoresis among protein extracts deriving from control or treated cells. After image analysis protein "spots" showing statistical difference in abundance between the two conditions were picked and identified with MALDI-TOF MS/MS. We found at least seventeen proteins upregulated during exposure of *L. lactis* to macedocin. Several of these proteins are known to be involved in the central metabolism like the pyruvate dehydrogenase E1 component alpha and beta subunits, the alcohol-acetaldehyde dehydrogenase, the inosine-5'-monophosphate dehydrogenase etc., while others are stress proteins like the ATP-dependent Clp protease proteolytic subunit, the superoxide dismutase, the toxic anion resistance protein etc. Additional proteins of interest were the GTP-sensing transcriptional pleiotropic repressor CodY, the two-component system regulator llrD and the cell division protein SepF. Our findings suggest a pleiotropic effect of macedocin on *L. lactis*. We are currently seeking validation of these observations at the transcriptome level.

P131 Proteomic analysis of the secretome from normal and malignant cervical cell lines

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Cervical cancer is the second most common malignancy in women worldwide causing 493,243 incidents and almost 273,505 deaths every year. Cervical cancer is attributed to high risk HPV types, mainly HPV16 and HPV18. The study was focused on the secretome of the cell lines as secreted proteins are an important source of potential biomarkers and pharmaceutical targets. The secretome of normal keratinocytes HCK1 (Human Cervical Keratinocytes) was compared to different cervical cancer cell lines [C33-A (HPV-), SiHa (HPV16+), HeLa (HPV18+)]. The secretome profile of SiHa and HeLa was analysed by 2D-gel electrophoresis and it was significantly different from the proteomic profile of the corresponding total cell extracts. Secreted proteins from the SiHa cell line were identified by mass spectrometry. Cathepsin D, a secreted protein was upregulated in SiHa cell line compared to control HCK1. This proteomic result is validated by Immunohistochemistry data, thus Cathepsin D is a potential biomarker for cervical cancer.

P132 Proteomic approach for identification and characterization of immunodominant antigens of Leishmania infantum associated with protection against canine leishmaniasis

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Leishmaniasis defines a cluster of protozoal diseases with diverse clinical manifestations. Visceral leishmaniasis (VL) caused by *Leishmania (L.) donovani and L. infantum/chagasi* is the most severe form of the disease. Infection with *L. infantum* represents 20% of the global human cases of zoonotic VL. Since dogs are the main domestic reservoir for human infection, control of canine leishmaniasis (CL) represents a promising measure for disease elimination. In the present study, we analysed the sera reactivity patterns of dogs either naturally infected with *L. infantum* (asymptomatic, n=11) or suffering from CL (symptomatic, n=5) against the whole parasite protein. Animals consisting the two experimental groups were appropriately selected in terms of age, gender, sex and clinical status. Two-dimensional (2D) Western blot analysis revealed significant differences between the two groups; a higher number of spots were detected when using sera of asymptomatic dogs in comparison to symptomatic confirming the CL immunosuppression. Twelve spots were recognized exclusively by the asymptomatic sera assigned by Coomassie blue brilliant-stained gel to corresponding proteins. Mass spectrometry analysis of these spots resulted in the identification of several proteins, whose putative functions and/or immunological properties were retrieved from *L. infantum* Genome Project database. Our results describe a specific IgG recognizing pattern associated with resistance to *L. infantum* infection (asymptomatic dogs) and this information could be exploited for new immune monitoring or vaccine development.

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Proteomic signatures in a mouse model of Mesial Temporal Lobe Epilepsy (MTLE) for therapeutic targeting

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Mesial Temporal Lobe Epilepsy is a prevalent drug-resistant epilepsy syndrome arising in the hippocampus. Disease etiology is poorly understood and effective therapeutic targets are still lacking. Herein, a mouse model assembling many features of human MTLE was implemented to investigate proteomic signatures in the hippocampus at 6 h after a local injection of **kainite** inducing *status epilepticus*. Mice were injected unilaterally in dorsal hippocampus with **kainite** (1 nmol/50 nL) or saline (controls). Hippocampi were isolated at 6h for protein extraction. Samples were analyzed with 2D-DIGE and MALDI-TOF-Mass-Spectrometry followed by bioinformatical analysis using the Mascot software (homology probability>95%, p value<0.05, fold change≥1.5). Gene Ontology Annotations for identified proteins were retrieved by GeneCodis3 software, whereas IPA software, KEGG and PANTHER Classification System were used for the Functional analysis. Forty one different proteins were detected as significantly changed. Following data mining and literature search, a number of specific pathways emerged as differentially expressed during epileptogenesis at 6h post-kainate injection. Biological functions found affected were: cycle of synaptic vesicles (SVs) (e.g.Eea1↑, Rab21↑, Erc2↓, Hspa8↑, Ehd3↑), cytoskeletal alterations (e.g.Crmp1↑, Dpysl2↑, Spna2↑, Cit↑, Baiap2↑), carbohydrate and lipid metabolism (e.g.Pygb↓, Dlat↑, Gpd1↑, Gpd2↑), mitochondrial functions (e.g.Atp5a1↑, Trap1↑, Dnm1l↑). Overall, several aspects of neurotransmission, hippocampal cytoarchitecture and metabolic functions appear to be significantly modulated. Significant alterations were also observed in mitochondrial functions including fission. Regulating the observed neurotransmission aberrations may facilitate the reversal of the neuronal hyperexcitability during the initial focal *status epilepticus*. Further enhancing the upregulation of mito-protective proteins could reduce the high levels of neuronal death.

P134

The role of arylamine N-acetyltransferases in microbial xenobiotic metabolism

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Arylamine N-acetyltransferases (NATs) detoxify environmental xenobiotics, including pollutants. Our genomic surveys indicate the presence of NAT homologues in microorganisms, particularly bacteria and fungi of potential ecological, biotechnological and/or bioremediative significance. Our investigations have focused on 13 NAT homologues of five mycotoxigenic ascomycetes, namely the corn pathogen *Fusarium verticillioides*, the wheat pathogen *F. graminearum*, the tomato pathogen *F. oxysporum* f.sp. *lycopersici*, the grain contaminant *Aspergillus flavus* and the model fungus *A. nidulans*. Elucidation of the enzymatic properties of those homologues has been possible via recombinant protein expression, followed by enzymatic activity assay, kinetic analysis and differential scanning fluorimetry. Of particular interest is the NAT1 homologue of *F. verticillioides,* demonstrated to enhance pathogenic fitness of the fungus via detoxification of 2-benzoxazolinone (BOA), an anti-microbial defense toxin produced by cereals. Growth of *F. verticillioides* is not compromised by exposure to BOA, and the compound induces NAT1 gene expression and enzyme activity, as demonstrated by quantitative RT-PCR, microarray analysis and biochemical assays with cell extracts. Moreover, phylogenetic analysis pointed to a distinct clade of *NAT1* orthologues characteristic of maize-associated *Fusarium* species. *F. graminearum* also has NAT1-mediated detoxification capability against BOA and survives exposure, unlike the remaining fungi that demonstrate minimal resistance to the compound. We are now expanding our investigation into the detoxification capabilities of microbial NATs, via cloning and functional characterization of *NAT* genes from bacteria representative of diverse environments, ranging from ultra pure water to highly polluted industrial sites. The *NAT* genes of biodegradative *Bacilli* and biotechnologically relevant *Streptomycetes* are also investigated.

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Functional and biochemical characterization of a juvenile hormone esterase-related gene in the moth Sesamia nonagrioides (Lepidoptera: noctuidae)

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Juvenile hormone (JH) plays major roles in the control of growth, development, metamorphosis, diapause and reproduction in insects. Degradation of JH in tissues and hemolymph of insects, is a major strategy by which juvenile hormone titers are regulated and JH esterase (JHE) enzyme has been thought to play key roles in this process. Previously we isolated and characterized a juvenile hormone esterase-related (*SnJHER*) gene in the moth *Sesamia nonagrioides* (Lepidoptera: Noctuidae). The size of its transcript was 1.84 kb and the predicted protein contained all five functional domains which are present in most esterases, proteases and lipases. In this study, functional gene-knockdown assays using RNAi showed that *SnJHER* is implicated in larval-larval, larval-pupal and pupal-adult molts of *S. nonagrioides*. In addition, heterologous gene expression in lepidopteran insect cell lines and baculovirus-mediated over-expression assays revealed the potential implication of this gene in the detoxification of JH-analogues. We propose a dual role for *SnJHER* in the regulation of *S. nonagrioides* development and the detoxification of JH analogue-based insecticides

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A novel SLC25 family member of mitochondrial carriers causes severe recessive neurological disease in mice

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Following a forward genetics approach through random mutagenesis, we identified a novel mouse model of severe autosomal recessive neurological disease. The symptoms start at 3 weeks of age characterized by ataxia, unsteady locomotion, episodic crises and growth retardation with severe disease progression that leads to lethality in the majority of the mice by the age of 3 months. Through genetic analysis, we identified a nonsense point mutation in the coding region of a novel gene member of the Solute Carrier Family 25 (SLC25) that introduces a premature stop codon and results in a loss-of-function protein. SLC25 members localize into the inner mitochondrial membrane where they shuttle a variety of metabolites across it, while mutations in SLC25 genes impair mitochondrial functions.

This novel member is highly conserved among species, but its function remains completely unknown. Our results confirm its mitochondrial localization by confocal and western blot analysis. Moreover, we identified that the wild-type 46 kDa SLC25 protein is predominantly expressed in the Central Nervous System in contrast to the mutant protein that is undetectable. We have also verified the causal role of this mutation in rescue experiments by expressing the human ortholog in transgenic mice. Our ongoing studies are focused on a) the identification of the primary site of lesion in order to align the brain pathology of the mouse model with that of similar human neurodegenerative diseases, b) the identification of mitochondrial dysfunctions, and c) the functional analysis of this novel SLC25 protein which constitutes a novel pathogenic target in neurological diseases such as ataxia.

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P137

Activation of inflammasome and IL-1 pathways in a genetic heart failure mouse model

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Mice deficient in the muscle-specific intermediate filament protein desmin develop mitochondrial defects in cardiomyocytes, leading to dilated cardiomyopathy and eventually heart failure. A spontaneous inflammatory response triggered by the end of the second week of lifespan initiates adverse myocardial remodeling and extensive fibrosis, associated with cardiac dysfunction. Cells infiltrating the inflamed desmin-null heart overexpress, among other potential modulators, osteopontin, that promotes cardiac fibrosis and dysfunction in this model.

Inflammasome formation and subsequent interleukin-1β (IL-1β) activation are triggered by dying cells in models of sterile inflammation, including myocardial infarction, contributing to tissue pathophysiology. To investigate whether components of the IL-1 and inflammasome pathway could communicate sterile inflammation in the desmin-null model we analyzed cardiac expression and histology before, at and after the onset of inflammation and found an up-regulation of the inflammasome components NLRP3, ASC, as well as of procaspase-1 and IL-1β in the inflamed hearts and infiltrates. Moreover, we found a considerable up-regulation of IL-1ra, the native IL-1β antagonist, observable even before the onset of inflammation, whereas the expression of the relevant anti-inflammatory IL-10 remained unchanged. Real-time PCR analysis of cardiac subpopulations revealed a preferential up-regulation of ASC in desmin-null cardiomyocytes, whereas IL-1β and its antagonist IL-1ra were overexpressed by infiltrating immunocytes and cardiac fibroblasts. The above indicate cardiomyocyte changes that may trigger the inflammatory response in desmin-null heart and a potential immunomodulatory role of cardiac fibroblasts.

P138 Identification of protein phosphatase-1 complexes that are regulated by cAMP-dependent phosphorylation and impact cardiac contractility

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Contractile dysfunction associated with heart failure has been attributed to aberrant sarcoplasmic reticulum (SR) Ca^{2+} cycling. Sequestration of Ca^{2+} into the SR is mediated by the sarco(endo)plasmic reticulum Ca^{2+} transport ATPase (SERCA2a), the activity of which is reversibly regulated by phospholamban (PLN). PLN is an inhibitor of SECA2a and relief of this inhibitory effect occurs upon PLN phosphorylation, enhancing cardiac relaxation. PLN phosphorylation is primarily mediated by protein kinase A (PKA), while its dephosphorylation is controlled by protein phosphatase-1 (PP1). Importantly, PP1 activity is regulated by two PKA-phosphoproteins, inhibitor-1 (I-1) and small heat shock protein 20 (Hsp20). The aim of our study was to investigate the molecular mechanisms controlling PP1 by I-1 and Hsp20 and their impact on PLN phosphorylation.
Using complementary *in vitro* binding studies, we identified a multi-protein complex centered on protein phosphatase-1 that includes its muscle specific glycogen-targeting subunit G_M and substrate PLN (PP1/G_M/PLN). G_M interacts directly and independently with both PP1 and PLN. Importantly, the PP1/G_M/PLN complex dissociates upon PKA phosphorylation, indicating its relevance to the □-adrenergic response of the heart. Thus, efficient control of PP1 activity by regulatory subunits represents another important functional mechanism influencing PLN dephosphorylation and cardiac contractility. Indeed, human genetic variants of I-1 or Hsp20 exhibit reduced binding and inhibition of PP1, indicating the significance of PP1-mediated protein complexes in the heart.

Our study provides novel insights into the mechanisms underlying the fine-tuned regulation of PP1, influencing SERCA2/PLN and cardiac function.

P139 Novel small molecule inhibitors of human RANKL targeting its trimerization

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Receptor activator of nuclear factor-κB ligand (RANKL), a trimeric tumor necrosis factor (TNF) superfamily member, is the central mediator of osteoclast formation and bone resorption. Functional mutations in RANKL lead to human autosomal recessive osteopetrosis, whereas RANKL overexpression has been implicated in the pathogenesis of bone degenerative diseases such as osteoporosis. Following a forward genetics approach, we have recently shown that a novel loss-of-function allele of *Rankl* with a glycine-to-arginine susbstitution at codon 278, causes severe recessive osteopetrosis in mice due to inhibition of RANKL trimerization. Furthermore, as G278 is highly conserved within the TNF superfamily, we identified that similar substitutions in TNF and B-cell activating factor (BAFF) also impaired trimerization and binding to cognate receptors, resulting in loss of biological activity. Notably, SPD304, a small molecule inhibitor of TNF trimerization, also binds and inhibits RANKL, suggesting similar inhibitory mechanisms. However, SPD304 displays high cell toxicity. Based on the trimeric structure of RANKL and its interaction with SPD304, novel small molecules were designed to abrogate RANKL trimer formation and biological function while also displaying lower toxicity. Of the 72 SPD304-like derivatives synthesized and tested, 9 displayed complete inhibition of human RANKL function in osteoclastogenesis assays with less cytotoxicity compared to SPD304 using MTT assays. Our results identified potent small molecule inhibitors of human RANKL designed to target and block its trimerization. The more effective inhibitors will be further evaluated in vivo using our unique human RANKL-expressing transgenic mouse models of osteoporosis.

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P140

Spontaneous development of hepatocellular carcinoma from ductal progenitor cells in PR-SET7 deficient mice

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Hepatocellular carcinoma, like other cancers, consist of phenotypically heterogeneous neoplastic cells. A subset of them referred to as Cancer Stem Cells (CSCs), exhibit unlimited self-renewal and multi-lineage differentiation capacity. Understanding the origin and supporting mechanisms of CSCs is of considerable significance. This study, conducted in liver-specific PR-SET7 knock-out mice, demonstrates that hepatic CSCs can be derived from normal progenitor cells located in the biliary ducts of the liver, which partially differentiate to hepatocytes and undergo oncogenic transformation. Hepatocyte-specific deletion of *PR-SET7* in embryonic liver resulted in G2 arrest followed by massive cell death and defect in liver organogenesis. Inactivation at postnatal stages caused cell duplication-dependent hepatocyte necrosis with unusual features of autophagy, termed "endonucleosis". Necrotic death was accompanied by inflammation, fibrosis and compensatory growth induction of neighboring hepatocytes and resident ductal progenitor cells. Prolonged necrotic-regenerative cycles coupled with oncogenic STAT3 activation replaced pre-existing hepatocytes with hepatocellular carcinoma derived entirely from ductal progenitor cells. Hepatocellular carcinoma in these mice displays a cancer stem cell gene signature specified by the coexpression of ductal progenitor markers and hepatic oncofetal genes.

P141

Aberrant nuclear accumulation or depletion of neurofibromin leads to mitotic defects in neural cells

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Neurofibromin, a RasGAP, is a regulator of cellular proliferation; yet its actions as a tumour suppressor may not be solely explained on its ability to de-activate Ras. Tumor suppressors must shuttle to the nucleus to control progression to mitosis. We have previously shown that an NES and an NLS on the primary sequence of neurofibromin are functional, as its subcellular distribution between cytoplasm and the nucleus is highly regulated during the cell cycle. In addition, neurofibromin's nuclear accumulation in late G2 phase is energy dependent and regulated by RanGTPase, as well as intense, PKCε-dependent phosphorylation on Ser2808, a residue localized by the NLS. We now show that overexpression of the C-terminal domain-CTD of neurofibromin, containing NES and NLS, led to nuclear accumulation of the recombinant protein in a plasmid dose-dependent manner. Moreover, when CTDSer2808 was mutagenized to the phosphomimetic Asp, nuclear accumulation was almost as quick as translation. Next, we examined the effect of CTD constructs on the proliferation rate of glioma cells and found that while CTDs increased the proportion of mitotic cells at first, in the long-term apoptosis prevailed, indicative of mitotic slippage due to a dominant negative effect. Indeed, lentiviral delivery of specific *NF1*-RNA interference led to quick reduction first of the nuclear neurofibromin pool, and more importantly, it resulted in a marked increase in mitotic aberrations and then in apoptosis in several cell lines. Taken together, our results indicate that nuclear neurofibromin actively participates in the mitotic process, a previously implied function of the protein.

P142 Ablation of Akt1 and Akt2 regulates mammary cancer progression by cell-autonomous and non-autonomous mechanisms in mice

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Most breast cancers overexpress and/or activate the serine-threonine kinases Akt. Our previous studies of Akt isoform-specific deficient mice have demonstrated that ablation of Akt1 inhibits while ablation of Akt2 accelerates the growth of mammary adenocarcinomas in Neu-transgenic mice. Because mammary adenocarcinomas are epithelial neoplasms, the high levels of Akt1 and the low levels of Akt2 in tumors arising in Neu/wild-type mice could be the result of preferential expression of Akt1 in epithelial cells and Akt2 in the stroma. However, some of Akt1-deficient Neu- transgenic mice regained tumor potential following a prolonged latency period. Tumors arising in these Akt1-deficient mice are more invasive than tumors arising in wild type and Akt2-/- mice. We show that Akt2 is activated in the epithelial tumor cells in response to Akt1 loss. The effects of Akt1- and Akt2-deficiency in oncogenesis by Neu could be either cell-autonomous or stroma-dependent. This issue was addressed by transplantation experiments. Akt1- or Akt2-deficient mammary epithelium expressing Neu was transplanted into wild-type stroma and host background as well as in Akt-deficient isoform-specific genetic background. Our results demonstrate that Akt1-deficiency delays tumor development independently of stroma genotype and indicate that the effect of Akt1- but not Akt2-deficiency on mammary epithelium during Neu-induced tumorigenesis is cell-autonomous. Since cancer and normal development share common mechanisms, we next performed transplantation experiments during pregnancy. According to our results, again only Akt1 plays a cell-autonomous role in the proliferation and differentiation of mammary secretory epithelial cells. Our studies provide insight in Akt1 and Akt2 function during cancer progression in either cancer cells or their environment.

P143 Biosynthesis and high-throughput genetic screening of cyclic peptides with potential therapeutic properties for Alzheimer's disease.

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Alzheimer's Disease *(AD)* is the most widespread neurodegenerative disease, its symptoms including cognitive and functional impairment while ultimately, the disease is terminal. The prevalent opinion as to the molecular cause of the disease involves the amyloid beta protein, a polypeptide of 42 residues with an inherent propensity for aggregation. The aggregates formed by amyloid beta 42 *(Aβ42)* cause toxicity to neuronal cells, leading to neurodegeneration and the onset of AD. The aim of this project is to identify small cyclic peptides that inhibit aggregation in neuronal cells thus possibly acting as therapeutic compounds for AD.

For this purpose,

- We constructed combinatorial libraries of random peptide sequences in *E. coli* cells using a technology that provides a large and diverse cyclic peptide library (10^7 individual peptides) and allows co-expression of this library with our target protein.
- We then used an easy and high-throughput bacterial screen for peptides that inhibit Aβ42 aggregation. This assay makes use of an Aβ42 fusion with a reporter protein to correlate proper Aβ42 folding with an easily monitored cellular phenotype.

By co-expression of the cyclic peptide library along with the Aβ42 in fusion with a reporter protein we have managed to discover and isolate several cyclic peptides that inhibit Aβ42 aggregation. These peptides are being studied in order to determine their potential therapeutic properties.

P144 Differential activation of TGF-β and BMP signaling pathways in renal fibrosis

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Renal fibrosis defined as the accumulation of abnormal amounts of extracellular matrix is the common end point for all progressive renal diseases. Although many signaling pathways are involved in fibrosis the pathophysiologic mechanisms are not yet clarified. Unilateral ureteral obstruction (UUO) is an important model for the study of mechanisms of renal fibrosis. Furthermore, the use of genetically engineered mice has greatly expanded the utility of the model. In this study, we report the use of TRED/BRE mice expressing Green Fluorescent Protein (GFP) under the control of BMP-responsive elemenet (BRE) and Red Fluorescent Protein under the control of TGF-β responsive element (TRED) in order to visualize the distribution of transcriptional activity of BMP and TGF-β signaling in healthy kidneys and in UUO model of renal fibrosis. TRED/BRE mice were sham-operated and ligated and were sacrificed at 2 and 8 days after ureteric ligation. In healthy kidneys, TGF-β transcriptional activity was mainly localized in podocytes and epithelium of distal tubules whereas the BMP transcriptional activity was restricted mainly to podocytes and collecting tubules. In the UUO model differential upregulation of both signaling pathways was observed: TGF-β in Henle's loop and distal tubules and BMP in distal and collecting tubules. Remarkably, in the UUO, phenotypically similar cells in close proximity and belonging to the same tubule exhibited all four possible activation states: both active, one or the other active, none active. The above data suggest a complex pattern of activation of these pathways in renal fibrosis requiring further studies.

P145 Expression and characterization of extracellular domains of MuSK for use as immunoadsorbents for the development of an antigen-specific therapy for myasthenia gravis

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Myasthenia gravis (MG) is an antibody-mediated autoimmune disease that affects the neuromuscular junction (NMJ). About 85% of MG patients have autoantibodies (autoAbs) against the muscle nicotinic acetylcholine receptor (AChR), while about 5-7% of patients have autoAbs against the muscle specific kinase (MuSK). This protein is important during the development of the NMJ and it is also expressed at the mature NMJ, where it mediates the agrin-induced clustering of AChRs. MuSK is a transmembrane protein which consists of an extracellular region, a transmembrane domain and an intracellular, tyrosine kinase, catalytic domain. The extracellular domain (ECD) consists of three immunoglobulin–like (Ig) domains and a cysteine-rich domain. The ECD carries most of the epitopes for MuSK autoAbs. Current treatments, including plasmapheresis, are non specific, causing several side effects such as the removal of indispensable plasma components in the case of plasmapheresis. We aim to develop an antigen-specific therapy in which only anti-MuSK auto-Abs will be removed from patients' sera using the immobilized MuSK extracellular domain (ECD) as immunoadsorbent. We have expressed the ECD of MuSK, with and without the cysteine-rich domain, in *Pichia Pastoris.* After immobilization on CNBr activated sepharose beads the MuSK-ECD columns were used as immunoadsorbents. MuSK-MG patients' sera were depleted from anti-MuSK autoAbs and the two columns compared to each other. The affinity of the anti-MuSK autoAbs, the recycling and the stability of the columns were examined. Our intention is to develop a safe antigen-specific MG immunoadsorption therapy with the minimum cost for the patient.

P146 Glucosamine sulfate stimulates glycosaminoglycan synthesis and diminishes the TNFα-mediated up-regulation of MMP-3 in nucleus pulposus intervertebral disc cells

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Glucosamine that naturally occurs in cartilage tissues has been widely used for treating osteoarthritis, but its role in the chondrocyte-like nucleus pulposus cells has not been thoroughly investigated. We have previously reported that glucosamine sulfate does not reverse the high osmolality-mediated delay of proliferation in nucleus pulposus cells needed for the maintenance of the tissue's homeostasis, while a long-term incubation with the molecule increased the cells' glycosaminoglycan content [Spine (Phila Pa 1976). 2013 Feb 15;38(4):308-14]. We followed by assessing the effect of glucosamine sulfate on nucleus pulposus cells' responses triggered by tumor necrosis factor α (TNFα) that constitutes an early signal of disc degeneration. TNFα was not found to be cytotoxic for nucleus pulposus cells in the concentrations tested and resulted in a ~2.5-fold increase of the intracellular reactive oxygen species (ROS) levels. The cytokine led to a rapid transient phosphorylation of the p38 MAPK and a ROS-dependent activation of the JNKs without affecting the phosphorylation levels of ERKs and Akt. In addition, TNFα had a prominent inflammatory effect on nucleus pulposus cells by up-regulating *mmp-3* expression, a phenomenon that was abolished when inhibiting the kinase activity of p38 MAPK. The induction of *mmp-3* mRNA levels was reversed to a degree after pre-treatment of the cells with glucosamine sulfate, but the pathways that participate in this phenomenon are yet to be elucidated. Overall, our findings support a possible promising clinical role of glucosamine (alone or in combination with other drugs) for treating or even better preventing disc degenerative disorders.

P147 HSP70 genetic deletion but not Glutamine administration alters cytokine expression in murine peritoneal macrophages following LPS stimulation

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INTRODUCTION: Glutamine (Gln) has been shown to protect against inflammatory injury and illness in experimental and clinical settings. The mechanism of this protection is unknown; however, recent data have indicated a relationship between Gln-mediated protection and enhanced heat shock protein 70 (HSP70) expression, as well as Gln-mediated inhibition of nuclear factor kB (NF-kB). Heat Shock protein (HSP) expression is vital to cellular and tissue protection following stress or injury.

OBJECTIVES: To clarify the mechanisms Gln utilizes to exert the beneficial effects on the inflammatory response and to determine the interaction between Gln and HSP70 during inflammation.

METHODS: Macrophages are the major source of producing and releasing proinflammatory cytokines. Gln metabolism is initiated by glutaminase, which catalyzes the conversion of Gln to glutamate and ammonia. Macrophages have very high glutaminase activity. Therefore, we used primary macrophages collected from wildtype mice (*Hsp70+/+)*and mice with genetic deletion of the *Hsp70* genes (*Hsp70-/-)*. Cells were treated with 100ng/ml LPS in the presence or absence of 10 mMGln. Cytokines were measured with ELISA. NF-κB protein expression was evaluated by Western blot.

RESULTS: Proinflammatory cytokine levels were significantly higher in *Hsp70-/-* compared to *Hsp70+/+* macrophages. Similarly, NF-κB protein levels were also higher in *Hsp70-/-* compared to *Hsp70+/+* macrophages. However, administration of Gln prior to LPS did not affect either cytokine or NF-κB protein levels in macrophages isolated from either genotype.

CONCLUSIONS: Our preliminary results indicate that HSP70 likely exerts a protective anti-inflammatory effect in macrophages. The mechanism of this effect is under further investigation.

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P148 Identification of signaling molecules that control a pro- and anti-inflammatory phenotype of adipocytes

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Obesity is a growing problem and a leading cause of diseases such as diabetes, insulin resistance and cardiovascular disease. The chronic low-grade inflammatory state that occurs in obesity is critical to the development of these obesity-induced pathologic conditions. Aim of our work is to elucidate the contribution of adipocytes in metabolic inflammation. Adipocytes share common features with macrophages such as the response to TLR ligands and the production of pro and anti-inflammatory mediators. Macrophages can obtain a pro-inflammatory phenotype (M1) or an alternatively activated phenotype that is mainly anti-inflammatory (M2) by differentially expressing the enzymes iNOS and Arginase 1 (Arg1). Whether adipocytes obtain a similar phenotype and express these enzymes is not known. Using RNAi screening and immunofluorescence analysis of 3T3L1 pre-adipocytes and mature adipocytes we monitored if and how knock-down of signaling molecules and transcription factors alters the inflammatory phenotype of these cells by measuring Arg1 and iNOS protein levels. We found that upon differentiation, 3T3L1 cells acquire an anti-inflammatory phenotype as observed by increase in Arg1 and decrease in iNOS levels. PPARγ, STAT3 and Akt-kinases appear to participate in controlling the pro- and anti- inflammatory phenotype of adipocytes.

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P149 In vivo activation of the nuclear receptor PPARbeta/delta attenuates cardiac remodelling and modulates autophagy in diabetic rat heart

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Diabetes is characterized by cardiac metabolic and contractile dysfunction and remodelling leading to heart failure. Accumulated evidence demonstrates a cardioprotective role for peroxisome proliferator-activated receptors (PPARs), as key transcriptional regulators of lipid and energy metabolism, in susceptibility to myocardial I/R injury. We investigated whether in vivo activation of PPARβ/δ, using the specific agonist GW0742, ameliorates cardiac contractile dysfunction and remodeling and restores metabolic impairment in streptozotocin-induced diabetic rats. We also explored the effect on autophagy, as dysregulated autophagy has been implicated in diabetic cardiomyopathy. PPARβ/δ activation attenuated diabetes- induced cardiac dysfunction and inhibited hypertrophy and fibrosis. Furthermore, treatment with GW0742 restored the diabetes-induced decreased expression and transport of glucose transporter GLUT4. Compared with control rats, diabetics exhibited increased expression levels of genes participating in fatty acid metabolism (PPARβ/δ, mCPT-1, MCAD) whereas treatment with GW0742 further enhanced these expression levels. Diminished autophagy in diabetic rats, as indicated by the reduced ratio of LC3II/LC3I and increased expression of p62, was restored by PPARβ/δ activation. Furthermore, AMPK, an activator of autophagic process, was inhibited in diabetic rats whereas treatment with GW0742 restored AMPK phosphorylation to control levels. In conclusion, PPARβ/δ activation compensates cardiac metabolism impairment and restores autophagy through AMPK activation, resulting in attenuation of cardiac remodeling in diabetic myocardium.

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P150 Loss of the tumor suppressor phosphatase PTEN reduces sensitivity of breast cancer cells to antiestrogens

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Luminal A breast cancer (LABC) expresses estrogen receptor (ER), progesterone receptor (PR), and very low levels of HER2 and responds to antiestrogens such as tamoxifen and fulvestrant. However, a fair percentage of LABC eventually develops resistance to antiestrogens due to PI3K mutation(s) and/or PTEN loss. The aim of the present study is to investigate the role of PTEN loss on the response of LABC cells bearing mutant *PIK3CA* (catalytic subunit of PI3K) to antiestrogens. To this end, we used shRNA to knockdown PTEN in T47D LABC cells and tested their sensitivity to tamoxifen and fulvestrant under full growth and insulin-deprived conditions. PTEN-deficient T47D cells showed significantly decreased ER levels and resistance to both antiestrogens in full growth conditions. In addition, knockdown of PTEN resulted in mild resistance to Lapatinib and GDC-0941, inhibitors of HER2/EGFR and PI3K signaling, but pronounced resistance to AEW-541, inhibitor of IGF-1R signaling. Resistance to these inhibitors was abolished in the absence of insulin. Experiments are in progress to analyse the mechanism of IGF-1R involvement in the development of acquired resistance of LABC cells to antiestrogens.

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P151 MiR-200 family miRNAs significant downregulation and miR-3069 overexpression is observed in the late mouse skin carcinogenesis stages

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MicroRNAs are short RNA molecules consisting of approximately 20-23 nucleotides and act as post-transcriptional regulators via binding to complementary sequences on target mRNAs and subsequently causing gene silencing. Aberrant expression of several miRNA molecules has been correlated with several types of cancer. However, its exact role in oncogenesis has not been elucidated yet. The multistage mouse skin carcinogenesis model is a well-documented system comprising of several cancer cell lines (i.e C5N, H-ras null, P1, P6, MSP5, PDV, PDVC57, B9, E4, A5, D3, H11 CarB and CarC) which represent distinct stages of chemically-induced skin carcinogenesis. In this study, we have performed microarray analysis in order to monitor miRNA expression in cell lines representing initiation, promotion and progression stages of mouse skin oncogenesis. We have detected alterations in specific miRNAs (downregulation of miR-200a, miR-200b, miR-200c, miR-429, miR-141 and overexpression of miR-3069) during progression from the normal and benign papilloma cells to the squamous and spindle malignant cells. Real Time PCR technique has been used in order to validate the results of this high-throughput analysis. Particularly, we have shown the significantly altered expression of the examined miRNA molecules between early and late stages of carcinogenesis. The five miRNAs of the miR-200 family are downregulated and miR-3069 is overexpressed in the aggressive, highly metastatic spindle cells in comparison with the early squamous carcinoma cells. Moreover, we observed a significant downregulation of miR-200b, miR-200c and miR-141 in a group of aggressive human breast cancer cell lines compared to the non malignant breast cell line MCF12A.

P152 Regulation of estrogen receptors' expression in young and senescent normal and tumor-associated breast human fibroblasts

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Estrogens are considered to be an important parameter in breast tissue development, homeostasis, as well as pathophysiology. Several breast tumors are estrogen-responsive, thus many anticancer therapeutic approaches focus on the regulation of estrogens' action. However, less attention has been devoted to the effect of estrogen on stromal cells surrounding the tumor. Accordingly, aim of the present work was to investigate the expression of estrogen receptors (α and β) in stromal fibroblasts derived from normal tissue and breast tumors, as well as the role of estrogens in stroma-tumor interactions. To this end, we have developed primary cultures from normal stromal fibroblasts and cancer-associated fibroblasts from consenting volunteers undergoing surgery. Our preliminary data indicate that, under in vitro conditions, stromal fibroblasts express more copies of ERβ mRNA than ERα. However, the expression of both genes was dramatically lower than that found in the breast cancer cell lines MCF-7 and MDA-MB-231. We did not observed any significant effect of short- and long-term exposure of fibroblasts to estradiol concerning their viability, proliferation and the expression of ERα and ERβ. It is widely accepted that the aged stroma affects significantly tumor progression. Accordingly, we have developed senescent breast fibroblasts after exposure to ionizing radiation and studied their characteristics. Our preliminary observations indicate that young and senescent fibroblasts express similar levels of ERα and ERβ. Finally, paracrine interactions of fibroblasts and cancer cells will also be discussed.

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P153 Transcriptional regulation of neurofibromin isoforms and potential differential functions in neurons

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Mutations in the *Nf1* gene lead to neurofibromatosis (NF1, von Reckilnghausen's) disease, which is characterized by aberrant growth of specific cell types and by learning disabilities, including autism. Its product, the RasGAP neurofibromin, is highly expressed in the CNS and increases in its expression correlate well with gains in differentiation and synaptogenesis. We have also identified and shown that neurofibromin contains a functional nuclear localization signal (NLS) in exon43, which may be spliced out as shown in screens of normal human tissues. We thus first investigated the expression of NLS and ΔNLS transcripts in CNS tissue and CNS-derived primary cultures and found that rat, mouse, or chick astrocytes possessed only +NLS, whereas in neurons ΔNLS transcript increased progressively with differentiation. Similarly, induction of differentiation in established human cell line models, correlated with induction of ΔNLS, as long-term treatment of SHSY-5Y neuroblastoma cells with retinoic acid almost abolished expression of NLS transcripts. *In ovo* long-term activation of PKCε, a signaling kinase known to induce differentiation, also increased ΔNLS. Next, in an effort to identify cellular functions of these two neurofibromin subpopulations using an array of cells expressing both or either transcripts, we found that ΔNLS-neurofibromin exhibited lower affinity for β-actin, an interaction that provides control over its RasGAP activity; and more importantly that it bound to and co-immunoprecipitated neuroligin 3, a postsynaptic density receptor, genetically linked to autism. These results report for the first time differential functions of the two neurofibromin subpopulations that may explain specific neuronal symptoms of the disease.

P154 A Novel Role of the Endoplasmic Reticulum (ER) in Transducing VEGF Signals that regulate Endothelial Cell Survival and Angiogenesis

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The endoplasmic reticulum (ER) is responsible for protein folding, maturation, trafficking and quality control becoming stressed when newly synthesized unfolded proteins accumulate in its lumen. ER stress initiates the IRE1α, ATF6 and PERK cascades leading to a transcriptional/translational adaptive response known as the Unfolded Protein Response (UPR). Here we show that VEGF, the key modulator of vessel formation, activates UPR mediators through a PLCγ-mediated cross-talk with the mTORC1 complex that is independent from the enzymatic activity of PLCγ on PIP2. Indeed, activation of UPR mediators was independent from IP_3/Ca^{2+} and DAG/PKC perturbations, but was blocked by rapamycin, an inhibitor of mTORC1 complex. Gene silencing experiments supported the results obtained with the inhibitors and additionally revealed that activation of ATF6 and PERK contributes to the survival effect of VEGF on endothelial cells (ECs) by positively regulating mTORC2-mediated phosphorylation of AKT at Ser473, which is required for full activity of AKT. Concomitant instability of the CHOP mRNA and protein allows ECs to evade the pro-apoptotic effect of this UPR product. Depletion of PLCγ, ATF6 or eIF2α inhibited dramatically VEGF-induced vascularization in mouse Matrigel plugs suggesting that the ER and the UPR machinery constitute components of the VEGF signaling circuit that regulates endothelial cell survival and angiogenesis. Filtering the VEGF signals through metabolic sensors (mTORC1) and the ER probably ensures that VEGF-dependent endothelial cell responses are compatible with the metabolic status of the endothelial cell.

P155 CRF (Corticotropin Releasing Factor) system in human heart fetal development

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The CRF system (neuropeptides CRF, Ucn I, II, III and binding sites CRF1, CRF2, CRF-BP) is responsible for stress regulation and homeostasis. Recent evidence from animal model studies involves the CRF system in the developmental process of different fetal organs and in parturition. In this study, CRF system localization was investigated using immunohistochemistry in 40 archival human fetal heart samples of different gestational age, deriving from spontaneous abortions or elective therapeutic termination of pregnancy. Samples were divided, according to diagnosis, in Group A (no diagnosed pathology), Group B (chromosomal abnormalities) and Group C (congenital disorders). The study protocol was approved by the Ethical Committee of the University Hospital of DUTH. Immunoreactivity for all antigens was found throughout fetal growth and was cytoplasmic and membranic (neuropeptides) or membranic (binding sites). Ucn III was more frequently present before the 21st gestational week (p=0.021) and in Group C than in Group A (p=0.016). In conclusion, both ligands and binding sites of the CRF system are present in the developing fetal heart and Ucn III seems to be correlated to early development and pathology. These results need further investigation, as Urocortins have also been involved in the adult heart pathophysiology and therapeutics.

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Evaluation of the antiestrogenic activity of new analogues of Raloxifene

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The Selective Estrogen Receptor (ER) Modulator (SERM) Raloxifene (Evista®) is used for prevention of breast and endometrial cancer as well as osteoporosis. SERMS such as Raloxifene and Tamoxifen (Novaldex®), antagonize the effect of estrogen in the mammary gland and the uterus while mimicking the protective effects of estrogen on the bone. Raloxifene, in particular, is preferably used for osteoporosis prevention since it is known to display low uterotrophic activity compared to Tamoxifen. We developed 18 new analogues of Raloxifene, 14 normal ones that contained different side chain modifications that nonetheless preserved the basic amine, benzyl and carbonyl moieties and 4 novel analogues that contained side chains with the basic amine and different cycloalkyl moieties substituting for the benzyl and carbonyl groups. The 18 analogues were evaluated for antagonism of estrogen action through ERα and ERβ using cell lines with estrogen-responsive reporter genes and for antiestrogenic effects in the breast and uterus using estrogen sensitive mammary and uterine adenocarcinoma cells in the presence of pro- and post-menopausal levels of estradiol (1 and 0,1 nM, respectively). We also evaluated the relative (to estradiol) binding affinity of the analogues for isolated ERα and ERβ. All the analogues displayed higher affinity of binding to ERα compared to ERβ, as was also the case with raloxifene and tamoxifen. The analogue with a cyclobutyl-containing side chain displayed 100-fold higher affinity of binding to ERα than to ERβ but was moderately antiestrogenic in breast and uterine adenocarcinoma cells. This was also the case with the remainder cycloalkyl analogues as well as with 12 of the normal ones. However, two of the latter analogues with bulky basic amines substituting for the piperidine side chain tip of raloxifene were strongly antiestrogenic, with the bulkiest tip-containing analogue being more antiestrogenic than raloxifene in breast and especially in uterine adenocarcinoma cells. The antiestrogenic activity of these analogues appeared to reflect their higher antagonist activity through ERα. Docking-scoring studies are in progress in order to assess the mode of binding of the two analogues to ERα.

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Evidence for the implication of human DDC isoforms in Docetaxel and Mitoxantrone induced cytotoxicity

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L-Dopa decarboxylase (DDC) is a pyridoxal 5-phosphate (PLP)-dependent enzyme that catalyses the decarboxylation of L-Dopa to dopamine (DA). It has been shown that the human DDC gene undergoes alternative splicing, leading to the production of different protein isoforms. Very little is known about the biological function of these isoforms. Although DDC expression has been implicated in the pathogenesis of many human cancers types, nothing is known about the role of human DDC alternative isoforms in cancer pathogenesis and disease progression. In this report, we have investigated the effect of full length DDC, human alt-DDC and DDC lacking exon 3 (DDC-3) expression, on chemotherapeutic agent - induced cytotoxicity. Transfected CHO cells, expressing the above human alternative DDC isoforms, were treated with Docetaxel and Mitoxantrone. Our results indicated that all DDC expressing CHO cells demonstrated increased levels of cytotoxicity in the presence to both agents, when compared to control CHO cells (**Docetaxel**: CHO:0%; CHO/DDC-Alt:10.2±1.2; CHO/DDC-3:17.3±1.7. **Mitoxantrone:** CHO:0%; CHO/DDC-Alt:16.4±1.1; CHO/DDC-3:34.6±3.1). Furthermore, our results indicated that both chemotherapeutic agents influence the expression of human DDC isoforms. The above data provide evidence for the participation of DDC isoforms in chemotherapeutic agent - induced toxicity. Further investigation of the findings presented here, aiming at the understanding of the role of DDC, as well as its isoforms, on the survival of cancer cells, might provide us with information that could lead to the comprehension of the emerging role of this complex molecule in the pathogenesis of neoplasia.

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The obesity-linked human homologues Twz (KCTD15) and TfAP2 (TFAP2B) regulate feeding behaviour in Drosophila

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Obesity and metabolic syndrome are increasingly endemic problems of modern societies. Although, Genome Wide Association Studies (GWAS) have identified a number of genes linked to obesity, for many of these genes the molecular function is still unknown. *KCTD15* and *TFAP2B* are two obesity-linked genes revealed by GWAS[i]. To gain an understanding of how these two genes regulate metabolism, we studied their *Drosophila* homologues, *Twz* (human *KCTD15*) and *TfAP-2* (human *TFAP2B)*. Interestingly, data obtained by SOLiD sequencing of the entire transcriptome where *Twz* and *TfAP-2* expression were disrupted revealed a disregulation of genes responsible for starch and sucrose metabolism. Of note, it was also discovered that overexpression of *TfAP-2* inhibits transcription of *eye transformer* (*et*), which acts as an inhibitor of the *Drosophila* JAK/STAT signalling pathway[ii]. Recently, it was observed that similar to the mammalian Leptin pathway, activation of the JAK/STAT pathway in GABAergic neurons leads to release of the *Drosophila* insulin-like peptides (DILPs) from the insulin-producing cells (IPCs) within the brain[iii]. By knocking down *Twz* and *TfAP-2* in specific regions of the *Drosophila* brain, and subsequently measuring the effect on lipid storage, feeding behaviour and activity, we demonstrate that these two obesity-linked homologues play a central role in the regulation of metabolism.

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P159 Dynamics and functional properties of the lamin B receptor (LBR) at the ensemble average and the single-cell level

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The lamin B receptor (LBR) has often been used as a marker of the inner nuclear membrane and as a reporter of nuclear envelope dynamics. However, what we currently know about the intracellular mobility of this protein is largely based on the properties of a "designer's mutant", which shows correct localization, but lacks the carboxy-terminal two-thirds of the molecule. Drawing a distinction from previous studies, we show here that the diffusional mobility of LBR exhibits region-specific variation, consistent with its non-uniform distribution along the inner nuclear membrane and the asymmetry of the nuclear envelope-endoplasmic reticulum interface. Interestingly, removal of transmembrane domains II-VIII (as in the "designer's mutant") lowers significantly the ensemble average mobility of LBR at the nuclear envelope, but does not affect much its mobility in the endoplasmic reticulum. On the other hand, deletion of transmembrane domains V-VIII renders the protein hyper-mobile. Over-expression of wild type or hyper-mobile LBR in embryonic stem cells lowers significantly the dynamic range of Nanog fluctuations and affects the expression of hundreds of genes without causing exit from the non-differentiated state. Lineage-specific defects are observed when cells over-expressing LBR or its mutants are induced to differentiate, suggesting a major role in developmental events.

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P160 Acetylation of polyamines is influenced by Cd2+-mediated stress

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Polyamines constitute an important component of the defense mechanism against various environmental stresses, such as metal-mediated stress. Recently, we demonstrated that exposure of mussels (*Mytilus galloprovincialis*) to seawater containing 100 μg/l Cd^{2+} for 15 days generated an oxidative-stress status, characterized by elevated production of superoxide radical, high levels of lipid peroxidation, and lysosomal membrane instability. Meanwhile, metallothionein levels increased gradually, while superoxide dismutase activity was elevated at day 5 and then declined. Similarly, free putrescine production was induced at day 5 and then declined. In contrast, free spermidine content was gradually reduced, whereas the concentration of free spermine increased. These results suggested that certain polyamines, like metallothioneins and superoxide dismutase, are an important component of the cell defense against oxidative stress induced by Cd^{2+}. However, several cellular acetyltransferases might have specifically acetylated free polyamines, converting them to substrates of APAO that subsequently cleaves them to generate polyamines of smaller size, thus effectively reversing the anabolic reactions. To obtain a complete picture of polyamine metabolism under Cd^{2+}-mediated stress, the levels of acetyl-polyamines were also measured. We observed that acetyl-spermidine increased concomitantly with the accumulated Cd^{2+} in digestive glands. In contrast, Cd^{2+}-mediated stress exerted on acetyl-spermine level an opposite effect. Acetyl-putrescine concentration, after a burst at the 5th day of exposure, followed a similar descent trend. Despite these changes, acetyl-polyamines were generally kept at low levels throughout the exposure period. Therefore, the increase in polyamine content must be attributed to induction of polyamine synthesis rather than inhibition of polyamine degradation.

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Desmin deficiency is linked to abnormal β-adrenergic receptor signaling

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Desmin, the muscle-specific intermediate filament protein, binds the TRIM-like protein, myospryn which is expressed in cardiac and skeletal muscle. Myospryn colocalizes with desmin at the periphery of the nucleus of mouse neonatal cardiomyocytes and at intercalated discs (IDs) and costameres of adult cardiomyocytes. Desmin is necessary for proper localization of myospryn around the nucleus and seems to play a role in biogenesis and/or distribution of lysosomes. Interestingly, myospryn is an AKAP protein (A Kinase Anchoring Protein) raising the possibility that together with desmin, it could participate in the subcellular targeting of cAMP protein kinase (PKA) activity. We want to specifically address the importance of desmin and myospryn-AKAP association in the regulation of PKA pathway, which is of particular importance for cardiomyocyte function. Under normal circumstances the activation of PKA pathway increases the rate and force of cardiac muscle contraction. However, during heart failure the myocardium becomes desensitized to β-adrenergic stimulation. On the contrary, prolonged β-adrenergic stimulation, through administration of its agonist isoproterenol, promotes ischemic-like cardiac injury. Importantly, β2-adrenergic receptors (β2-ARs) are restricted to specialized caveolar regions, where desmin has been found to colocalize and associate with caveolin-3. To determine whether desmin cytoskeletal network plays a role in β2-adrenergic signaling, neonatal cardiomyocytes from the desmin -/- mouse model of dilated cardiomyopathy, were treated or not with isoproterenol. Desmin deficiency affects at least the beating rate, cyclic-AMP accumulation and plasma membrane localization of β2-ARs, suggesting a potential role of desmin and myospryn-AKAP association in proper β-AR/adenylate cyclase coupling and desensitization.

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Idas is implicated in ciliogenesis during multiciliate cell differentiation

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A strict balance between proliferation and differentiation is essential for normal development to occur. Idas, a novel protein related to the cell-cycle regulator Geminin, is a bifunctional protein with roles in regulating both DNA replication and multiciliate cell differentiation.

Idas (a cousin of Gemini in ancient Greek mythology) is conserved in vertebrates and bears a coiled-coil with significant similarity to the coiled-coil domain of Geminin, through which the two proteins form a tight heterodimer. Idas depletion from cells affects cell cycle progression and leads to cell accumulation in S phase. These cells cannot proceed to Mitosis and G1. On the other hand, Idas overexpression results in an increase in DNA content and causes abnormal multipolar spindle formation and multinucleated cells.

Idas has an additional role in regulating cell differentiation. During mouse embryonic development, Idas exhibits high expression levels in the developing ciliated epithelia and specifically in the choroid plexus and the respiratory epithelium. Ex vivo experiments using a mouse tracheal epithelial cell culture system, reveal that Idas is expressed to high levels at the beginning of multiciliation, whereas FoxJ1, which marks the muticiliated cells during and after ciliogenesis, is highly expressed later than Idas. Multiciliated cells no longer express Idas at the end of the differentiation process, suggesting that Idas is required for the establishment but not the maintainance of the multiciliated cell fate. These results implicate Idas as an early regulator of multiciliogenesis.

P163 Selective adhesion and maintenance of circular shape of articular chondrocytes on Si surfaces patterned with poly (vinyl alcohol)-based photosensitive film

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Chondrocyte implantation is important for treatment of cartilage defects; however, chondrocytes are de-differentiated to fibroblast-like cells when cultivated in monolayers, thus limiting their therapeutic use. Chondrocyte de-differentiation is manifested both by the loss of cells round shape and the significant reduction in typical fibrocartilage ECM components expression such as collagen II and aggrecan. Here, we employed a Si substrate photolithographically patterned with poly(vinyl alcohol) (PVA) film and we investigated its ability to guide the adhesion of de-differentiated chondrocytes so as to retain their round shape and the possible effects it causes on cells re-differentiation. The substrates were prepared by spin-coating of phosphotungstic acid ($H_3PW_{12}O_{40}$)/PVA solution on Si wafer to create a thin film which was then exposed through a photomask to deep UV. Following development, the non-exposed PVA film areas were removed and revealed circular Si areas on the substrate with diameters of 10-100 μm and pitches of 30-300 μm, surrounded by cross-linked PVA. Results obtained after 8 d cultivation of chondrocytes (10^5 cells/mL) on these substrates indicated that they adhered selectively on Si areas, acquired circular shape, and proliferated unhindered when the circles diameter was 50 μm with 100 μm pitch. The chondrocyte shape maintenance on re-differentiation was evaluated through determination of the aggrecan gene expression by RT-PCR and aggrecan accumulation by immunofluorescence. It was found that aggrecan gene expression was 4 times higher and aggrecan concentration 8-20 times higher for cells cultured on the patterned substrates compared with those grown on plain Si or standard culture dishes. Thus, Si patterned through PVA film could be a promising substrate to prevent chondrocytes de-differentiation during culture.

P164 IGF and EGF signaling crosstalk on breast cancer cell adhesion

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Breast cancer (BC) is a heterogeneous tumor, with genotypic and phenotypic diversity. It has been established that Insulin-Like Growth Factor-I (IGF-I) and Epidermal Growth Factor (EGF) signaling pathways, with implications on crosstalk, regulate processes correlated to tumorigenesis of breast cancer. Moreover, the expression of estrogen receptors is associated with the mortality of BC patients. The aim of this study was to investigate the role of IGF-IR in the IGF and EGF signaling pathway on BC cell adhesion. In this study, we used three BC cell lines, of different estrogen receptor (ER) status (MCF-7: ERa+/ERb-, ZR-75-1:ERa+/ERb+, MDA-MB-231:ERa-/ERb+). We examined the effect of IGF-I on IGF-IR expression and on cell adhesion in all BC cell lines. MDA-MB-231 cells express extremely low levels of IGF-IR, in contrast to high levels in MCF-7 cells. IGF-I negatively affected the IGF-IR mRNA expression in MCF-7 and in ZR-75-1 cells ($p<0,01$). IGF-I did not affect the adhesion capacity of MCF-7 and MDA-MB-231 cells but it stimulated cell attachment of ZR-75-1 cells onto fibronectin ($p<0,01$). EGF stimulates MCF-7 ($p<0,01$) and ZR-75-1 ($p<0,001$) cell adhesion. The inhibition of IGF-I signaling pathway, via IGFR blocking, decreased the basal ($p<0,05$) but significantly inhibited EGF-induced cell adhesion of MCF-7 cells ($p<0,001$). Moreover, the inhibition of IGF-IR decreased the effect of EGF($p<0,001$) and IGF($p<0,01$) on adhesion of ZR-75-1 cells. Our results suggest that the Insulin-Like Growth Factor –I Receptor (IGF-IR) plays a significant role in breast cancer cell adhesion and in the action of IGF and EGF on BC cell adhesion.

P165 Mitoprotection by αB-crystallin overexpression: a key mechanism in the rescue of desmin-deficient heart failure

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Desmin and the small heat shock protein αB-Crystallin are major targets in dilated cardiomyopathy and heart failure. Their association combined with the fact that mutations in either one of them lead to heart failure in humans and mice, suggests a potential compensatory interplay between them in cardioprotection. To address this hypothesis we investigated the consequences of the αB-Crystallin overexpression in a desmin deficient (des-/-) genetic model of heart failure.

We generated des-/- mice overexpressing αB-Crystallin in the heart (des-/-αBCry) and demonstrated by echocardiography significant improvement of cardiac function to almost wild type levels, correlating to 100% survival rate in an obligatory exercise swimming protocol during which only 50% of des-/- mice survive. Des-/- ultrastructural defects, such as mitochondrial abnormalities and myofibril disarray have been corrected to a significant degree, reflecting almost complete rescue of cell death, fibrosis and myocardial degeneration. αB-Crystallin overexpression provides significant protection against increased endogenous levels of ROS found in des-/- adult cardiomyocytes, consistent with the αB-Crystallin-dependent increase in glutathione levels. Moreover, we found that mitoprotection by αB-Crystallin overexpression is linked to maintenance of proper mitochondrial protein levels, inhibition of abnormal activation of the mitochondrial permeability transition pore (mPTP) and the decrease of mitochondrial membrane potential ($\Delta\psi$) in the absence of desmin. Furthermore, we found a potential link between desmin, αB-crystallin and mitochondrial morphology and bioenergetics through their interaction with mitofilin and ATP synthase.

In conclusion, αB-Crystallin overexpression rescues extensively mitochondrial defects and cardiomyocyte death due to desmin deficiency, inhibits heart failure development and could be a potent therapeutic target for this disease in humans.

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P166 Multiple roles of Integrin-Linked Kinase in follicle epithelial cells in Drosophila

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Drosophila oogenesis is a genetically tractable system suitable to investigate various developmental processes involving actin dynamics and cell adhesion. Each female has two ovaries consisting of ovarioles, each of which can be effectively considered as an egg chamber production line, containing egg chambers of different developmental stages. The germarium is located at the anterior end of the ovariole, containing somatic and germline stem cells, followed by egg chambers that bud off and mature, reaching the posterior side where eventually become eggs competent for fertilization.

Integrin_Linked Kinase (ILK) has been shown to act in concert with integrins to regulate cell-matrix adhesion. It has also been shown that ILK is implicated in actin dynamics. So, in order to study the role of ILK during oogenesis in *Drosophila*, we used two complimentary approaches. First, we generated female flies lacking ILK from the entire ovary besides the interfollicular stalk cells. We found that ILK is indispensable for the early stages of oogenesis. Loss of ILK disrupts the stalk cell formation and the separation of the successive newly formed egg chambers in a non-cell autonomous manner. Moreover, the developing eggs fail to elongate properly, indicating a requirement for the follicle epithelium morphogenesis. Second, we generated clones of mutant cells within the developing ovary, which allowed us to investigate the role of ILK at later developmental stages. This analysis revealed that ILK regulates primarily the organization of actomyosin in the follicle epithelial cells. Collectively, our data uncover novel regulatory functions for ILK in the developing epithelium.

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Study of membrane-associated human l - dopa decarboxylase alternative isoforms

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L-3,4-dihydroxyphenyl-alanine decarboxylase (L-DOPA decarboxylase, DDC, EC 4.1.1.26) decarboxylases L-DOPA to dopamine. L-DOPA decarboxylase was considered to be a cytosolic molecule. Recent studies, from our laboratory, have shown the association of DDC with cellular membranes in *Ceratitis capitata*, mouse brain and human cells, as well as the solubilization of DDC from human cells. However, little is known about the subcellular topology and solubilization of human DDC alternative isoforms. In this study, we investigated the membrane association of human alt-DDC and DDC lacking exon 3 (DDC-3) and aspects of solubilization of DDC-3. Transfected CHO cells, expressing the above human alternative DDC isoforms where used in these experiments. Treatment of transfected CHO cells with the amphiphilic detergent Triton X-114, revealed the detection of DDC-3 protein molecules in all three separation phases, whereas the alternative human isoform alt-DDC was recovered only in hydrophilic and highly hydrophobic phases. Our results, indicated the solubilization of membrane-associated human DDC-3 in a pH and time-dependent manner. Furthermore, our data showed that only a portion of the membrane-associated DDC-3 molecules were released into the soluble fraction. The solubilization of DDC-3 was affected by the presence of divalent cations, underlining the complexity of the mechanisms that mediate the release of the human truncated protein from cellular membranes. The findings presented here, indicate the membrane association, as well as the solubilization of human alternative DDC isoforms in CHO cells, highlighting the intricate regulatory mechanisms of this complex molecule.

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Subcellular localization of the human Glutamate Dehydrogenase homologues

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More than 99% of mitochondrial proteins are nuclear encoded, synthesized in the cytosol and imported in mitochondria via different pathways that ensure their correct targeting. Glutamate Dehydrogenase (GDH) is an enzyme present in almost all living organisms and plays a central role in the cellular metabolism such as energy and ammonia homeostasis, metabolism of glutamate and neuroprotection. GDH is involved in a number of human disorders such as epilepsy, Parkinson's disease and the HI/HA syndrome. In humans GDH exists in the highly homologous isoforms, hGDH1 and hGDH2 that differ in their tissue-distribution and allosteric regulation properties. We studied the subcellular localization of human GDH isoenzymes and the importance of their N-terminal cleavable presequence for their appropriate targeting using fluorescence microscopy and fractionation in mammalian cell lines as well as radiolabelled protein import assays in isolated yeast mitochondria. We observed that the human GDHs are localized mainly in mitochondria. Both human GDH proteins can be imported in isolated yeast mitochondria and cleaved in their appropriate mature from in a mitochondrial inner membrane potential ($\Delta\psi$) and a metal dependent manner. The N-terminal cleavable presequence of both proteins acts as a leader-peptide for their mitochondrial localization. Last but not least, the first predicted alpha-helix of the hGDH2 has an essential role in this process.

P169 The ILK/Parvin/PINCH-complex stabilizes the integrin adhesome and modulates integrin avidity

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The linkage of integrins to cytoskeleton is mediated by the integrin adhesome, a network of interconnecting cytoplasmic proteins. The tripartite protein complex containing Integrin Linked Kinase (ILK), Parvin and PINCH, namely IPP-complex, is one of the most well conserved integrin adhesome modules. We have previously shown an essential function of the IPP-complex in the maintenance of the integrin-actin link. Here we combined genetic analysis and live-imaging of muscle attachment sites in *Drosophila* embryo, to analyse how the molecular organisation of the adhesome influences integrin attachment to the extracellular matrix (ECM). We observed, in IPP mutants, an initial integrin detachment from the ECM and a concomitant disintegration of the adhesome network, while F-actin retained its association to adhesome members at muscle attachment sites. Progressively, integrin adhesion became deteriorated and actin filaments detached. Gain-of function point mutations in the extracellular domain of β_{PS} integrin subunit that enhance integrin affinity were sufficient to restore largely, both integrin adhesion to ECM and intracellular organisation of the adhesome network. However, the linkage of F-actin to integrins was not rescued. Collectively our data reveals additional two novel interdependent functions of the IPP-complex in the living organism; it stabilizes the integrin adhesome and increases integrin avidity for ECM ligands.

P170 The role of cross-talk between ERs and growth factors receptors in cell localization of heparan sulfate proteoglycans in breast cancer cells

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Heparan sulfate proteglycans (HSPGs) play a diverse role in tumor biology by mediating cell adhesion and migration. Syndecans constitute one major category of transmembrane HSPGs, which regulate cell-cell and cell-extracellular matrix (ECM) interactions. Recent studies have revealed new insights into ER action in breast cancer, highlighting the role of an intimate cross-talk between the ER, epidermal growth factor receptor (EGFR) and insulin growth factor receptor (IGFR) signaling pathways in the development of resistance to endocrine therapies against the ER pathway. The aim of this study is to elucidate the localization of SNDs and ERs in the presence and the absence of E2 and the role of EGFR/IGFR pathways in localization of these molecules. For this purpose, two breast carcinoma cell lines MCF-7 (ERα+) and MDA-MB-231 (ERβ+) were cultured in the presence and the absence of E2 after pre-treatment with EGFR and IGFR inhibitors. As a result, the localization of SNDs and ERs seems to be regulated by E2 and ER status of breast cancer cells. Specifically, in ERα+ cells SND-4 is localized in nucleus and cytoplasm in genomic pathway of ERs, in contrast with ERβ+ cells where both molecules are co-localized in cytoplasm. Moreover, in non-genomic pathway SND-4 and ERs are co-localized perinuclear in both cell types. EGF/IGF signaling pathways seem to regulate the expression of SNDs in both ERs pathways. Finally, our study highlights that the use of EGFR and IGFR inhibitors in combination or separately may be a promising therapeutic tool for HSPGs-mediated metastatic potential in cancer microenvironment.

Acknowledgements

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P171 The role of RAS, BRAF and PIK3CA oncogenes in cancer cell apoptosis and autophagy

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Cancer cells develop resistance to therapeutics through modulation of key apoptotic and autophagic pathways. RAS pathway has a central role in a high percentage of tumour development. RAS and BRAF oncogenes rarely co-exist in a single human neoplasm, but they can appear together with PIK3CA mutations. They present both similar and differential impact on tumour properties, including the modulation of key apoptotic pathways.

RAS and BRAF oncogenes are drivers of tumour development and their role in apoptosis and autophagy has been the subject of intense research interest. Thus, the differential role of RAS and BRAF oncogenes, and their cross-talk with PIK3CA in cancer cell apoptosis and autophagy has been addressed in this study.

The impact of individual RAS/BRAF oncogenes on tumour development includes modulation of key apoptotic pathways, like TRAIL and BCL2 family. This provides cancer cells with altered sensitivity to anti-cancer agents. Selected RAS/BRAF oncogenes can provide cells with EMT and autophagic properties, which can affect tumour cell survival and altered sensitisation to therapeutics.

We have used isogenic tumour cell systems with modified RAS, BRAF and PIK3CA oncogenes in 2D and 3D culture, mouse xenografts and clinical tumour specimens bearing oncogenic RAS/BRAF mutations. Key oncogene driven intrinsic and extrinsic apoptotic and autophagic pathways have been analysed. Their impact on sensitisation/resistance to cancer therapeutics has been examined.

P172 Unravelling the transport mechanism of fungal amino acid transporters: the paradigm of the PrnB

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The PrnB proline transporter of *Aspergillus nidulans* is a well characterized member of the APC superfamily Homology threading, using the crystal structures of bacterial amino acid transporters LeuT and AdiC, has produced a model of the PrnB structure. This structure shares a fold commonly found in many transporters, consisting of two intertwined, antiparallel V-shaped repeating units (TMSs1-5 and TMSs 6-10) connected by a relatively long loop (21-29 residues long), followed by two additional helices (TMSs11 and 12). In LeuT and AdiC, TMSs 1, 3, 6 and 8 contribute to substrate binding. Mutations in TMS1 of PrnB have previously confirmed its involvement in substrate translocation. In this study, we extend our knowledge in the PrnB structure-function relationships. More specifically, using *in vitro* site-directed mutagenesis, direct radiolabelled proline uptake measurements and competition assays, we further confirm that residues in TMS1 contribute to substrate binding and also identify residues in TMSs3, 6 and 8 that are necessary for the functionality of PrnB and/or contribute to the determination of its kinetic characteristics and specificity profile. Moreover, in a fully functional PrnB allele devoid of Cys residues, we apply cysteine scanning mutagenesis in residues located in TMS6 in order to further confirm their proximity to the substrate binding pocket.

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This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: THALES, Investing in knowledge society through the European Social Fund.

P173 Quantitative changes in Focal Adhesion Kinase regulate secretory signaling for cell survival

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Focal adhesion kinase (FAK) is a major component of the integrin-linked focal contacts and is implicated in a variety of physiological cellular functions including motility, invasion and anoikis. Whilst FAK overexpression is a feature of various tumor types and contributes to carcinogenic processes, genetic studies in mice demonstrate that FAK-depleted epithelial cells remain susceptible to malignant transformation through mechanisms which remain ill-defined. Herein we demonstrate that silencing of FAK in tumor cells activates an NF-□B-dependent signaling pathway which promotes cell survival by sustaining an IL-6/STAT3 auto-regulatory loop. Thus, the knock-down of FAK in cervical and lung cancer cell lines leads to accelerated I□B□ degradation, increased translocation of RelA/p65 NF-□B to the nucleus and NF-□B transactivation. These effects are accompanied by elevated IL-6 mRNA and protein levels which become normalized upon p65 NF-□B knock-down. Production of IL-6 leads to the activation of STAT3 and enhanced anchorage-independent growth. Silencing of FAK also promotes NFκB-dependent expression of cIAP2 which operates as a survival factor in part by augmenting NFκB signaling. Double silencing of both FAK and cIAP2 restores IL-6 levels and STAT3 phosphorylation to normal levels. SMAC mimetics which induce cIAP degradation act in concert with FAK ablation to reduce tumor cell viability. Our results reveal a novel function of FAK and indicate therapeutic opportunities by targeting specific aspects of FAK signal transduction.

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P174 Unveiling the dynamics of multi-protein complexes of DNA damage response pathways following irradiation in living cells

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Detection of structural DNA aberrations caused by genetoxic lesions leads to the formation of damage-specific and highly dynamic multi-protein complexes on sites of damage, which provide signals locally for repair and globally for cell cycle arrest and/or apoptosis. The precise spatio-temporal proteolysis of cell cycle regulators ensures control over cycle progression and genomic stability both in damaged and undamaged cells. Cdt1 has a crucial role in DNA replication licensing and DNA damage responses. We show that following localized ultraviolet irradiation or double-strand break formation by nanosurgery using a pulsed laser, Cdt1, the Cul4-DDB1^{Cdt2} E3 ubiquitin ligase component Cdt2 and the replication/repair factor PCNA accumulate at sites of damage with rapid kinetics. Cdt2 accumulation is independent of Cdt1 and persists following Cdt1 proteolysis. In order to elucidate the hierarchy of this signaling cascade and the kinetics of the implicated proteins, protein recruitment quantification analysis and fluoresence recovery after photobleaching (FRAP) experiments were conducted in transiently transfected live cells. FRAP experiments were then analyzed using easyFRAP and recruitment dynamics were quantified with custom ImageJ macros. We have constructed and analysed mutant forms of Cdt1 and Cdt2 in regions required for mutual and PCNA interactions, as well as for modifications by cell cycle and DNA-damage response specific kinases, such as MAP and ATM/ATR kinases. These mutants exhibit differential recruitment and FRAP kinetics. Comparative analysis of mutant proteins provides information in time and space of the post-damage interactions of Cdt1 and its binding partners within the cell nucleus and how these are integrated with DNA damage responses.

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Investigation of Cdt2 recruitment kinetics following localized DNA damage

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The maintenance of genome integrity is of major importance for cells. Targeted proteolysis is central for cell cycle control. E3 ubiquitin ligases govern cell cycle transitions by regulating the levels of key cell cycle regulators. These ligases catalyze the ubiquitination of specific protein targets which are then targeted for degradation. $CRL4^{Cdt2}$ belongs to this family and is responsible for regulating the levels of the licensing factor Cdt1 during S phase and post DNA damage. Set8 and p21 are also its substrates.

In order to study the regions of Cdt2 which are important for its function, we made two truncated versions of the protein, one containing the N-terminal part and another one containing the C-terminal part of the protein. We also generated mutant forms of Cdt2 which contain mutations in a PIP box important for interactions with PCNA and in 6 SQ sites important for phosphorylation via the ATR kinase.

In our experiments we induced localized DNA damage to MCF7 cells using micropore filters and UV irradiation in order to assess the recruitment of Cdt2 to the sites of damage. The recruitment analysis unveiled differences in the accumulation of the mutants at the sites of damage due to differences in protein interactions that take place after UVR. Fluorescence Recovery After Photobleaching (FRAP) was used in order to study dynamic interactions of Cdt2 and its mutant forms at sites of localized UV-damage and unravel how the different regions of Cdt2 contribute to protein-protein interactions mediating localized ubiquitination at sites of damage.

P176

Study of the RecQL4 family orthologue Hrq1 in fission yeast

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Genome integrity is essential for survival. To protect their genome, cells have evolved efficient DNA repair processes. RecQ family helicases play a key role in maintaining genome stability through their implication in DNA damage responses and are conserved through evolution. In humans, five RecQ-related helicases exist and mutations in three of them are linked to syndromes characterized by cancer predisposition and premature aging. RecQL4 is linked to Rothmund-Thomson premature aging syndrome and it is unique amongst RecQ helicases in being required for the normal initiation of DNA replication. Lower eukaryotic genomes encode fewer members of the RecQ family, simplifying the study of their evolutionarily conserved functions.

In fission yeast, two RecQ paralogues exist, Rqh1 and Hrq1, orthologues of the human BLM and RecQL4, respectively. To study the role of the RecQL4 orthologue in DNA replication and repair, strains bearing disrupted, epitope-tagged, GFP-tagged and inducible versions of Hrq1 were constructed. Hrq1 is not essential for cell survival, though Hrq1Δ strains exhibit slow growth following sporulation. Sequence analysis shows that Hrq1 shares an N-terminal domain with similarity to the DNA replication licensing factor Cdt1. In contrast, human RecQL4 contains a domain similar to the DNA replication factor Sld2. These fusion events between domains present on different proteins in different species suggest that Hrq1/RecQL4, Cdt1 and Sld2 (called Drc1 in fission yeast) may function as a complex. The interactions of Hrq1 with Drc1, Cdt1 and other replication initiation factors are examined by co-immunoprecipitation using strains expressing tagged versions of these proteins in cycling cells and following DNA damage.

P177

Parvin is required for egg chamber development in Drosophila

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Drosophila ovary is composed by developing egg chambers containing a 16-cell germline cyst, which is surrounded by a monolayer of epithelial cells. The follicle epithelium is derived from two follicle stem cells (FSCs) that reside in a specific structure called germarium. Despite of the phenomenal simplicity of the germarium, a complex sequence of signaling and adhesive interactions between somatic and germline cells is required for egg chamber epithelial formation and patterning. Previous studies have shown that integrin-mediated cell matrix adhesion plays important role in several aspects of egg chamber development, including a) maintenance of the FSCs niche, b) orientation of the mitotic spindles in dividing follicle epithelial cells, c) global tissue rotation and oscillating contractions of basal actomyosin cytoskeleton. The last two processes control the egg chamber elongation.

Here, we focus on the role of parvin, a conserved member of the integrin adhesome network. Parvin is highly expressed in specific cell populations of the follicle epithelium that play an essential role in follicle epithelium formation. To study the potential involvement of parvin in oogenesis, we used two complementary genetic approaches. First, we generated genetic mosaics and found that parvin is required to maintain the cell shape and position of prefollicle cells during the encapsulation process in the germarium. This phenotype bears a striking resemblance to the integrin mutant cells. Second, we generated mutant flies that do not express parvin in the entire ovary and observed a range of abnormalities including fused egg chambers with improper size and rounded shape.

P178

Analysing DNA re-replication in fission yeast at the single-cell level

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In every cell cycle DNA has to be copied accurately before mitotic division. Genome duplication starts from discrete sites along the genome, called origins of replication. Cells have developed multiple mechanisms to ensure that origins fire once during S phase and passively replicated origins are no longer able to fire, thus preventing any region from replicating again. Mutations that override these controls are found in different species and are linked to tumorigenesis. In fission yeast, cells that over-express Cdc18/Cdc6 undergo re-replication, accumulating a DNA content several fold higher than a wild type strain. Previous studies based on microarray analysis of populations have shown that the increase in DNA amount is not homogeneous along the genome, but some genomic loci are amplified to higher levels than others. We study the effect of re-replication in fission yeast at the single cell level using imaging techniques. We use cells that overexpress Cdc18 and also express the lac inhibitor (LacI) fused to GFP, which binds to specific regions of the genome tagged with several copies of the lac operator (LacO). Using the intensity of the GFP signal as an estimation of the DNA amount, we compare the different levels of amplification in single cells tagged in the same region or different regions along the genome. *In silico* data, derived from simulating a stochastic hybrid model of DNA re-replication, are used to predict how re-replication will proceed genome-wide in individual cells and are compared to experimental data for specific genomic loci. Our analysis reveals key features of DNA re-replication.

P179

Expression of Deadenylases as Prognostic Marker in Lung Cancer

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Lung cancer is a leading cause of cancer death worldwide. Non-small cell lung cancer accounts for 80–85% of all cases and it is further distinguished to squamous cell carcinoma (SCC), adenocarcinoma and other subtypes. The deregulation of mRNA stability is well established in lung cancer cells, and may lead to altered expression of oncogenes and/or tumor suppressors, suggesting that RNA metabolism-related molecules are involved in the development of the disease. Among these factors, deadenylases erode poly(A) tails, catalyzing the first and rate limiting step of mRNA degradation. Recent work from our lab has shown that the expression of deadenylases is altered in acute leukemias. Herein, we study the expression of deadenylases in SCC clinical samples and matched non-pathological ones. Bioinformatics analyses using published microarray data and qRT-PCR results from clinical samples show that the expression of several deadenylases is altered in SCC. We find that the expression of PARN and NOC deadenylases correlates with overall survival. To gain evidence of the pathways that might be affected by deadenylases, we silence their expression in human cells of SCC origin (NCI-H520) and study the impact on gene expression using microarrays, and on specific reporter genes using qRT-PCR. The analyses revealed distinct gene sets and pathways deregulated by the knockdown experiments. Our observations, suggest that the expression of specific deadenylases might be used as a prognostic marker in squamous lung carcinoma. They also indicate pathways and processes as targets for therapeutic approaches in the future.

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Analysis of C/EBPα hepatic transcription function

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Hepatic transcription factors regulate all aspects of liver organogenesis and physiology. The CCAAT/enhancer-binding protein α (C/EBPα) is a conserved transcription factor expressed from early developmental stages in the liver of multiple vertebrate species. We address the role of C/EBPα in the liver using the mouse as a model system. Inactivation of C/EBPα in the embryonic liver results in aberrant liver morphology and perinatal death of the knock-out animal, while inactivation in adult stages results in a less pronounced phenotype. We seek to identify the transcriptional targets of C/EBPα in the liver through combined micro-array, RNA sequencing, and genome-wide occupancy studies in various developmental stages. Our results reveal that C/EBPα directly regulates a broad number of hepatic genes across development. More importantly, our data support that in addition to functioning as a typical transcription factor, C/EBPα may also play a bookmarking role in the liver. This latter term describes a mechanism of early C/EBPα recruitment to promoters in embryonic life, generating and maintaining an open conformation of the locus for a prolonged period of time, allowing for activation later on in the life of the organism.

P181 (A knock-down approach reveals) a potential role for DNMT1 in cell cycle regulation during terminal erythroid cell differentiation mediated at the level of RNA pol II pausing.

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DNA methylation in mammals is conferred by the DNA methyl transferases (DNMTs) which include the DNMT3a and DNMT3b *de novo* methyltransferases and the DNMT1 maintenance methyltransferase which methylates newly synthesized hemimethylated strands of DNA following replication. Genomic DNA methylation patterns are highly dynamic during development and differentiation and have been implicated in various biological and developmental processes and in disease, e.g. cancer. Erythropoiesis is the generation of mature red blood cells from the HSCs through a multistep, tightly regulated process. Recent work from the Strouboulis lab provided evidence for the interaction of DNMT1 with key erythropoietic transcription factors, including the GATA-1 master transcription factor and its co-factors. These observations raise the prospect that DNMT1, and DNA methylation in general, may play an important role in erythroid differentiation. To address a potential implication of DNMT1 in erythroid cell differentiation, we performed DNMT1 shRNA knock-down experiments in mouse erythroleukemic (MEL) cells. Cytospin preparations, FACS analysis and expression profiling in three independent experiments showed a clear arrest in terminal erythroid differentiation with defects in cell cycle arrest which, at a molecular level, was manifested as a failure of repression of cell cycle related genes in the absence of DNMT1. Significantly, data meta-analysis provided evidence that cell cycle gene repression is mediated at the level of RNA pol II promoter pausing in cell cycle related genes. Thus, our data raise the prospect of DNA methylation repressing cell cycle gene expression at the level of RNA pol II pausing during terminal erythroid differentiation.

P182 Cell type specific regulation of glucocorticoid receptor transcriptional activity by Prox1

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Glucocorticoid receptor (GR) and Prox1 are transcription factors that are involved in many biological processes such as cell metabolism, growth and differentiation. The gene encoding the gluconeogenic phosphoenolpyruvate carboxykinase (PEPCK) enzyme is a well-known GR target, whose expression is also proposed to be regulated by Prox1. In this study the possible role of Prox1 in the transcriptional regulation of GR in HeLa and neuronal mN(euro)2A-Prox1 cells, stably transfected to conditionally expressed Prox1, was examined. Luciferase-reporter gene assay showed GR transcriptional activation by Prox1, even in the presence of GR agonists (dexamethasone, DEX) and antagonists (prednisolone, RU486) in neuronal cells, whereas Prox1 suppressed the GR transcriptional activity in HeLa cells, in the presence or absence of DEX or DEX and RU486. Luciferase-reporter gene assay also showed that none of the Prox1 DNA-, nuclear receptors-, and corepressors- binding domains is responsible for the Prox1 suppressive effect on GR transcriptional activation, in HeLa cells. Immunoprecipitation analysis revealed GR and Prox1 interaction in mN(euro)2A-Prox1 cells. Additionally, decrease in PEPCK expression by Prox1, in the presence or absence of DEX and RU486, in mN(euro)2A-Prox1 cells was observed by immunocytochemistry and Western blot analysis, which might indicate an independent GR and Prox1 action on PEPCK gene expression. Overall, our results indicate a cell-type specific GR transcriptional regulation by Prox1, which might be exerted though a direct or indirect GR-Prox1 interaction. We suggest that this interaction possibly leads to differential cell-type specific recruitment of regulatory molecules in the transcription initiation complex, and transcriptional regulation of target genes.

P183

CK1δ regulates HIF-1 heterodimerization as shown by in vivo and in situ analysis

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HIF-1 is a key regulator of the cellular response to hypoxic conditions. It acts as a heterodimer, consisting of two subunits, HIF-1α and HIF-1β (or ARNT). ARNT is constantly expressed while stability of HIF-1α is regulated by oxygen levels. HIF-1α is additionally controlled by oxygen-independent mechanisms including activating of its C-terminal part by ERK and modification of Ser247 at its N-terminal part by CK1δ that impairs HIF-1 transcriptional activity. Previous biochemical and in vitro experiments have suggested that phosphorylation by CK1δ reduces the affinity of HIF-1α for ARNT (Kalousi et al., 2010). To corroborate these results, we have applied fluorescence recovery after photobleaching (FRAP), fluorescence resonance energy transfer (FRET) and in situ proximity ligation assay (PLA) methods to study the formation of the HIF-1α/ARNT complex in living or intact cells under conditions that stimulate or inhibit CK1δ-dependent modification of HIF-1α. Analysis of cells expressing GFP-tagged forms of HIF-1α with FRAP shows that a Ser247 to Ala247 mutation or treatment with a CK1δ-specific inhibitor slows down the nuclear migration of HIF-1α indicating increased formation of a DNA-binding complex. This hypothesis is further supported by in situ PLA in cells grown under hypoxia (1% O_2), which shows that complex formation by endogenous untagged HIF-1α and ARNT is impaired when CK1δ is over-expressed but enhanced when CK1δ is inhibited. Taken together, our data demonstrate that phosphorylation by CK1δ controls in vivo HIF-1α/ARNT heterodimerization.

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Cobalt stimulates HIF-1-dependent but inhibits HIF-2-dependent gene expression in liver cancer cells

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Hypoxia-inducible factors (HIFs) are transcriptional regulators that mediate the cellular response to low oxygen. Although HIF-1 is usually considered as the principal mediator of hypoxic adaptation, several tissues and different cell types express both HIF-1 and HIF-2 isoforms under hypoxia or when treated with hypoxia mimetic chemicals such as cobalt. However, the similarities or differences between HIF-1 and HIF-2, in terms of their tissue- and inducer-specific activation and function, are not adequately characterized. To address this issue, we investigated the effects of true hypoxia and hypoxia mimetics on HIF-1 and HIF-2 induction and specific gene transcriptional activity in two hepatic cancer cell lines, Huh7 and HepG2. Both hypoxia and cobalt caused rapid induction of both HIF-1α and HIF-2α proteins. Hypoxia induced erythropoietin (EPO) expression and secretion in a HIF-2-dependent way. Surprisingly, however, EPO expression was not induced when cells were treated with cobalt. In agreement, both HIF-1- and HIF-2-dependent promoters (of PGK and SOD2 genes, respectively) were activated by hypoxia while cobalt only activated the HIF-1-dependent PGK promoter. Unlike cobalt, other hypoxia mimetics such as DFO and DMOG activated both types of promoters. Furthermore, cobalt impaired the hypoxic stimulation of HIF-2, but

not HIF-1, activity and cobalt-induced HIF-2α interacted poorly with USF-2, a HIF-2-specific co-activator. These data show that, despite similar induction of HIF-1α and HIF-2α protein expression, HIF-1 and HIF-2 specific gene activating functions respond differently to different stimuli and suggest the operation of oxygen-independent and gene- or tissue-specific regulatory mechanisms involving additional transcription factors or co-activators.

P185 Coup-TF and Embryonic Neural Development in the Sea Urchin

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Our aim is to identify the role of the orphan nuclear receptor PlCoup-TF in embryonic neurogenesis and especially in the determination of the anterior neuroectoderm (ANE). To this end, we cloned a set of embryonic cDNAs encoding regulatory proteins expressed specifically in the ANE and studied their spatial pattern of expression in conjunction with the expression pattern of PlCoup-TF. Thus, we prepared antisense RNA probes for fluorescence *in situ* hybridization for the following genes: *PlCoup-TF, PlSix3, PlHbn, PlNk2.1, PlFoxQ, PlZ81* and *PlFoxG* and perfomed double fluorescent *in situ* hybridization. The embryonic expression pattern of each gene in combination with PlCoup-TF was analyzed in all embryonic stages of the sea urchin *Paracentrotus lividus*. ANE, a neurogenic territory specified within the oral ectoderm of the embryo, is formed as a result of the interplay of the aforementioned regulatory factors that together constitute a sub-circuit within the embryonic gene regulatory network (GRN). We want to determine PlCoup-TF's place within the GRN and specifically the ANE sub-circuit. Therefore, we knockdown PlCoup-TF expression during embryogenesis by injecting specific morpholino antisense oligonucleotides (MASO) into sea urchin eggs and determine the expression pattern of the ANE specific genes by *in situ* hybridization to resulting morphant embryos. The efficiency of the knockdown is measured by qPCR, where the amount of PlCoup-TF transcripts is compared to control embryos. The effect of the specific PlCoup-TF MASO to embryonic neuronal development, will be assessed by using antibodies to neuronal markers, such as synaptotagmin and serotonin and immunofluorescence with the perturbed embryos.

P186 Development of an episomal vector or beta-globin gene that efficiently trasnfects mammalian cells

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Episomal vector are non-viral, non-integrating vectors of gene transfer, that circumvent the problem of insertional mutagenesis, presented by viral vectors. We present a new vector (16 Kb) carrying the HBB (human β –globin), whose transcription is driven by the β –globin promoter and the micro LCR (locus control region). The vector contains the reporter gene eGPF (enhanced, green fluorescent protein) and two chromosomal elements: (a) the S/MAR (scaffold and matrix attachment region), deriving from the 5" end of the human β –interferon gene, to facilitate plasmid retention in the host nucleus and is basic part of the prototype episomal vector pEPI1; and (b) the IR (initiation of replication), deriving from the 5" end of the β –globin gene, to enhance plasmid DNA replication. The new vector was successfully delivered into K562 cells (human, chronic myeloid leukemia cell line) by electroporation and nucleofection, in triplicate experiments that were kept in long term culture. Transfection efficiency, estimated by flow cytometry 24 h post transfection, was high in all cases, reaching 55% of cells. The expression of the eGFP, documented by fluorescent microscopy in the stable cell line after the addition of the antibiotic G418, is detected for 20 weeks of continuous culture. Importantly, it is also detected in paraller cultures that were kept without addition of the antibiotic G418, used for selection pressure. These are highly promising initial conditions for the development of an pEPI 1 based, episomal human β –globin vector for the gene therapy of the Haemoglobinopathies.

P187

Differential effect of the inflammatory mediators TNF-α, IL-6 and IL-1β on the expression and transcriptional activity of Hypoxia Inducible Factors HIF-1 and HIF-2 in human hepatoma cells

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Hypoxic tumour microenvironment and inflammation are common features in hepatocellular carcinoma (HCC). Whereas inflammation in combination with hypoxia trigger molecular events that facilitate tumor initiation, progression and metastasis, relatively little is known about the crosstalk between HIFs and inflammatory factors. Here, we investigate the expression and transcriptional activity of HIF-1α and HIF-2$^{\alpha}$ in response to the pro-inflammatory cytokines TNF-α, IL-6 and IL-1β in a human hepatoma cell line (Huh7) expressing both HIF-α isoforms. The expression of the hypoxia target genes PGK and EPO as well as plasmids expressing *PGK* or *SOD2* promoter constructs were used to specifically monitor HIF-1 or HIF-2 transcriptional activity, respectively. Our results show that HIF-1α and HIF-2α protein expression levels were not affected by any of the tested cytokines under normoxia. However, under hypoxia HIF-1α protein levels were decreased by TNF-α, increased by IL-6 and were not influenced by IL-1β. In contrast, none of these inflammatory mediators affected significantly the expression levels of hypoxia-induced HIF-2α. No effect on hypoxic induction of PGK or EPO could be observed when cells were treated with IL- 6 or IL-1β. On the other hand, TNF-α significantly reduced hypoxic induction of the HIF-2 specific gene, EPO, while it did not affect expression of PGK, a HIF-1 specific gene. In conclusion, we demonstrate for the first time that pro-inflammatory cytokines modulate differentially the expression and transcriptional activity of HIFs in HCC derived cells. This probably involves distinct molecular mechanisms, the investigation of which can improve our knowledge on carcinogenesis and contribute to better therapeutic strategies.

P188

Differential expression of p53-like isoforms in Mytilus galloprovincialis haemic neoplasia

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Mussels of the genus Mytilus can develop a leukaemia-like disease, haemic neoplasia in which the molluscan p53 tumor suppressor gene family was previously shown to be involved. Previous results of our laboratory revealed a putative p53 binding site in the promoter region of the *Mytilus galloprovincialis* hsp90 gene. We report here the first results on the expression of hsp90 and p53-like isoforms in *M. galloprovincialis* mussels with haemic neoplasia compared to normal ones. According to microscopical observations six of the 100 individuals collected from Thermaikos Gulf (Chalastra Bay, Thessaloniki-Greece) were found to be leukemic. Total RNA was extracted from gills and haemolymph of normal and leukemic samples and cDNAs were synthesized. Semi-quantitive PCR was performed to determine the expression level of hsp90 and p53, DNp63/73 and TAp63/73 isoforms. Both the p53 and the DNp63/73 isoforms were found to be up-regulated in neoplastic individuals, while mRNA levels of hsp90 and the TAp63/73 isoform did not change significantly. Expression of p53 was also characterized using a commercially available polyclonal antibody designed to detect human p53. Preliminary results of Western blot analysis showed a significant increase in expression of an approximately 80 kDa protein, assumed to be p73, in leukemic mantle tissues. However, no measurable difference was observed for a ≈50kDa protein expected to be p53 in normal and leukemic tissues. In conclusion, our results are in agreement with previous published data concerning molluscan species and give strength to our future work on the nature of interactions among stress response and apoptotic mechanisms.

P189 The role of p16INK4a in the degenerative phenotype of senescent human intervertebral disc cells

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Low back pain is considered one of the major chronic, age-related pathologies. It is associated with the degeneration of the intervertebral discs (IVD), the joints of the spine. We have previously shown the presence of a significant number of senescent cells in the aged and degenerated IVD. Having in mind the inflammatory/catabolic nature of senescent cells, it is believed that they participate in the degeneration of this tissue. The cell cycle inhibitor $p16^{INK4a}$ is responsible for the permanent growth arrest of senescent cells. However, previous studies indicated that it is not involved in the increased expression of inflammatory cytokines in senescence.

Here, we investigated the role of $p16^{INK4a}$ in the senescent phenotype of IVD cells, and especially in the expression of genes implicated in extracellular matrix homeostasis. To this end, Nucleus Pulposus (NP) and Annulus Fibrosus (AF) IVD cells were infected with lenti-virus carrying the $p16^{INK4a}$ gene under the control of a CMV promoter and thus they were rendered senescent. Senescent NP cells exhibit elevated levels of MMP9 mRNA and reduced levels of Aggrecan and ADAMSTS5 mRNAs, while senescent AF cells also exhibit elevated levels of MMP9, MMP13, Collagen II, and reduced levels of Aggrecan and Collagen I mRNAs. Similar results have been obtained from senescent cells after replicative exhaustion of exposure to ionizing radiation.

These results indicate that the overexpression of $p16^{INK4a}$ promotes senescence in IVD cells, followed by a catabolic phenotype, suggesting an important role in the integrity of extracellular matrix and the degeneration of this tissue.

P190 Effect of estradiol on proteasome activity and expression of its subunits in breast cancer cells

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Proteasome is a large intracellular multi-subunit protease complex that selectively degrades intracellular proteins. The transcription factor Nrf2, under certain conditions, has been shown to regulate the expression of proteasome β5 subunit. It has also been reported that estrogen significantly increases proteasome activity in microglial cells, and in breast cancer cells the estrogen-induced degradation of ERα is mediated by proteasome.

When the breast cancer cell line MCF-7 (expresses ERα) was cultured in the presence of estradiol, it was found that estradiol at concentrations 20-100 nM caused suppression of expression of β5 and β1 subunits of proteasome as well as of Nrf2, while at concentrations 1 and 10 nM it had no effect. The expression of β2 proteasome subunit was not affected by estradiol. In contrast, when the breast cancer cell line MDA-MB-231 (expresses ERβ) was cultured in the presence of estradiol, it was found that estradiol at concentrations 1 and 10 nM caused suppression of expression of all proteasome subunits as well as of Nrf2, while at concentrations 20 and 50 nM it enhanced the expression or had no effect. The proteasome activity was not significantly affected by estradiol in both cell lines.

In conclusion, the expression of proteasome subunits in both cancer cell lines may be mediated by Nrf2 activation. The observed differences between the two cell lines may be due to different ER expression in each cell line and could be correlated with the effect of estradiol on PI3-K, which is key molecule in Nrf2-mediated expression of antioxidative proteins.

P191

EGFR-/HER2-depended membrane metalloproteinases expression and functional properties of colon cancer cells

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Colorectal cancer (CRC) is the third leading cause of cancer-related death in the western world. Coexpression of EGFR and ErbB2 is found to a subset of colon cancer and may cooperatively promote cell survival, as heterodimerization is known to provide for diversification of signal transduction. Targeting either EGFR or ErbB2 combinatorially, has addictive or even synergistic antitumor activity, in colon cancer cells. In this study, we investigate the role of EGFR signaling in invasion and migration of Caco-2 colon cancer cells. The study is also focused on characterization of signaling pathways which mediate the EGFR gene expression, as well as matrix macromolecules' mRNA level. In particular RT-PCR analysis in Caco-2 cell line shows that EGF upregulates the expression of EGFR, but downregulates the gene of HER2 and as a result there is a balance between those two receptors. As far as concerned, the matrix macromolecules expression it seems that HER2 is responsible for the regulation of MT2-MMP, whereas EGFR signaling for the MT1-MMP gene expression. Moreover, concerning the functional properties of Caco-2 cells it is shown that the invasiveness of EGF stimulated cells is inhibited in the presence of EGFR's inhibitor, in a significant level. Intracellular inhibition of EGFR-mediated pathways regulates the migration of Caco-2 cells and downregulates the gene expression of MT1-MMP and EGFR, through the mitogen-activated protein kinase (MAPK) pathway. Conclusively, targeting the ErbB family may establish a clinical strategy of colon cancer therapy.

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P192

Evaluation of estrogenic activity of aluminum in breast cancer cells

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Aluminum-based compounds are the active ingredients in antiperspirants. Research on the effects of their long term use, especially in relation to the breast, which is a local area of application, revealed a potential role of aluminum salts in breast cancer development. Aluminum is also proposed to interfere with estrogen receptor actions. Given that estrogen receptors play a crucial role in breast cancer development, the metalloestrogenic potential of aluminum chlorohydrate (ACH) in breast cancer cells was examined. For this purpose, the possible differential effect of ACH on the transcriptional activation and subcellular localization of the two types of estrogen receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) was examined, in a dose dependent manner, in ERα-positive MCF-7 cells, MDA-MB-231, and HEK293 cells transiently transfected either with ERα or ERβ expression vectors. Studies on the effects of ACH on the expression of ERα target genes and cell growth were also performed. Our results revealed ACH estrogenic activity through both ERα and ERβ. ACH similar to E2 caused increase in the perinuclear localization of the cytoplasmic localized ERβ in MDA-MB-231 cells, whereas, the predominant nuclear localization of ERα did not significantly altered in the presence of either E2 or ACH, in MCF-7 cells. Our results also showed that ACH caused increase in cell proliferation, and regulate p53 and ERα expression in MCF-7 cells possibly through estrogen receptor actions. Taken together, our findings indicate that ACH possess potent estrogenicity that may, at least partly, account for its involvement in breast cancer development.

P193 Evaluation of the EGFR and HER2 regulatory roles in the expression and activity of the catalytic proteasomal subunits in colon cancer cells

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Colon cancer is the third, most common type of cancer worldwide. EGFR controls vital cellular processes, while his action is associated with poor prognosis. In colon cancer, dual EGFR/HER2 inhibition increases the anticancer impact of EGFR. Mutations in signaling molecules downstream of EGFR are very common in colon cancer. KRas and BRaf mutations are associated with the promotion of the disease and the anti-EGFR resistance.
The Ubiquitin-Proteasome system plays a crucial role in cancer, modulating important cellular processes and the activation of various transcription factors, such NRF2. A variety of studies have demonstrated that, the proteasomal inhibition causes cancer cells apoptosis. Furthermore, in some cases that inhibition has the ability to affect the action and the protein levels of EGFR. Nevertheless, there is no evidence if the reversed option is possible.
The aim of this study was, therefore to investigate the impact of EGFR and HER2 on gene expression and the activity of the catalytic proteasomal subunits, as well as to evaluate whether NRF2 plays a role in that process. Three colon cancer cell lines were used: Caco-2 (wild-type KRas and BRaf), DLD-1 (KRas mutated), HT-29 (BRaf mutated). Our results suggest that, EGFR can significantly regulate the expression and the activity of the proteasome, a process in which EGF significantly contributes. In addition, the dual EGFR/HER2 inhibition reduces both mRNA levels and the activity of the catalytic proteasomal subunits. Finally, in some cases inhibition of EGFR increases NRF2 mRNA levels, possibly as a response to EGFR- mediated proteasomal inhibition.

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P194 EZH2 histone methyltransferase co-operates with RAS signalling to regulate anoikis, EMT and tumour metastasis. Integrin a2 is a novel EZH2 target

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Interplay of genetic and epigenetic events are associated with cancer progression. Epithelial-Mesenchymal Transition (EMT) has impact on plasticity and tumorigenesis, and has been associated with metastasis. EZH2 is overexpressed in aggressive cancers, yet the mechanisms underlying this are largely unknown. We provide evidence supporting the idea that oncogenic Epithelial-Mesenchymal Transition is partially controlled by epigenetic factors such as EZH2. Evaluation of EZH2 mRNA and protein levels revealed overexpression in cell lines with metastatic traits. Analysis of EZH2 mRNA expression was expanded in clinical samples of colon cancer, and high level of EZH2 correlates with appearance of metastasis.

Another part of the study examines transcriptional regulation of EZH2 by signalling pathways. Inhibition of RAS regulated ERK and AKT in metastatic colon cancer cells attenuates EZH2 expression. EZH2 promoter analysis illustrates presence of AP-1 binding sites and occupancy of FRA-1 and C-JUN was demonstrated on EZH2 promoter.

Finally, novel EZH2 target genes have been identified, associated with cell migration. Abrogation of EZH2 expression impairs the ability of colon cancer cells to move, associated with anoikis in three-dimensional environment. Integrin a2 (ITGA2) was identified as novel EZH2 target by chromatin immunoprecipitation of EZH2, short hairpin RNA analysis and H3K27me3 occupancy changes on ITGA2 regulatory elements, associated with ITGA2 gene expression.

Interplay of genetic and epigenetic mechanisms regulate tumour aggressiveness. Activation of RAS regulated ERK/AKT pathways and FRA1/C-JUN induce EZH2 overexpression, which results in ITGA2 silencing and tumour cell anoikis. EZH2 is proposed as a metastasis marker and/or target for cancer treatment.

P195

From zygotic gene activation to wing patterning. A pluripotent transcriptional regulator of the Drosophila development

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In *Drosophila*, the transcription factor Zelda (Zinc-finger Early Drosophila Activator) binds specifically to promoters of the earliest zygotic genes and primes them for activation. Later in development Zelda is shown to be essential for patterning body structures such as the wing. In embryonic wing disc tissue of L3 larvae, *zelda'*s primary transcript is alternatively spliced into three transcript variants. In the same tissue two protein isoforms of ~180 and 70 kDa are detected. The unexpected 70 kDa isoform is probably a proteolytic product of one of the expected polypeptides and could have a distinct molecular function. A set of data presented here, support the hypothesis of considering Zelda as an essential and unique coordinator of gene activity throughout the development of *Drosophila*.

P196

How C/EBP recognition sites on a bidirectional promoter regulate the expression of Bombyx mori chorion gene-pairs

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Silkmoth chorion genes are arranged in divergently transcribed α/β gene pairs, sharing a common 5' flanking promoter region. These bidirectional promoters contain a complete set of cis-elements responsible for developmentally accurate gene expression. Noticeably, small divergence in their architecture, which involves the number of and distance between transcription factor recognition sites, is considered crucial for temporal specificity.

In this report we investigate the contribution to temporal gene specificity of four C/EBP binding sites contained in an early-middle promoter (pL9). Several constructs of pL9 bearing mutations on the above cis-elements were cloned upstream of the lacZ reporter gene in both orientations, so that the lacZ gene stood in for either the α- or the β- chorion gene. These were used in an electroporation-based transient expression method for ex vivo developing follicles. Constructs containing the complete promoter were used as control. Mutation of the C/EBP1 site which lies next to the α-gene, resulted in an earlier expression pattern only in the orientation where the reporter gene replaced the distal β-gene. Likewise, mutation of the C/EBP4 recognition site, which is closer to the β-gene, produced an earlier expression pattern only in the orientation where the lacZ gene replaced the distal α-gene. Furthermore, our preliminary results indicate involvement of the C/EBP2 site in the correct temporal expression of the α-gene and of the C/EBP3 site in the accurate expression of the β-gene.

We believe that these four C/EBP sites on the L9 promoter interplay to regulate temporal expression of α- and β- chorion gene-pairs.

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P197 Intracellular accumulation of heme signals nuclear localization of Nrf2 and transcriptional activation of stress response genes during the early phase of K562 cell hemoglobinization

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Hemin (Fe^{+++}-protoporphyrin IX) is the oxidized form of heme, an essential constituent of life and key-regulator of erythropoiesis. We have investigated the mode of intracellular accumulation of hemin during the early phase (0-12 hr) of hemin-induced differentiation of K562 CML cells and explored the question whether the entry of hemin induces transcriptional activation of Nrf2-driven stress response genes as well as the gene encoding the heme exporter Flvcr1a isoform. The expression of Gγ-globin served as specific erythroid marker. The amount of heme transported into K562 cells under varying concentrations of hemin (the pool of intracellular heme) was determined spectrophotometrically by using the pyridine hemochromogen assay. qPCR analysis revealed that hemin-treated cells transactivate Nrf2-driven genes (Heme-Oxygenase-1, Glutamate Cysteine Ligase, Thioredoxin and Ferroportin-1), which encode for metabolic enzymes that contribute to cell survival, intracellular reducing agents and for the only known iron exporter. The activation of stress response genes occurred via nuclear translocation of Nrf2, which takes place prior to Gγ-globin induction and reduction of Bcl11a transcriptional repressor protein level. Since the cytosolic extracts contain hardly detectable quantities of Nrf2, we concluded that hemin-induced biosynthesis of Nrf2 protein is accompanied by nuclear translocation within the early hours of treatment. Hemin-uptake, however, did not transactivate the FLVCR1a gene. These findings uncover molecular events that may occur upon the release of heme into the human damaged tissue in different disorders.

P198 INTRACELLULAR LOCALIZATION OF SERINE-ARGININE PROTEIN KINASE 1 (SRPK1) UNDER DIFFERENT METABOLIC STIMULI

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Serine-arginine protein kinases (SRPKs) are a subfamily of serine-threonine kinases that specifically phosphorylate serine residues located in regions rich in serine-arginine/arginine-serine dipeptide motifs, known as RS domains. SRPK1 was the first serine-arginine protein kinase to be purified and characterized. The first known and basic role of SRPK1 was the regulation of mRNA splicing. More recently however, it is well established that SRPK1 is involved in other cellular activities, such as chromatin reorganization, cell cycle regulation and metabolic signaling. Additionally, SRPKs emerge as interesting pharmaceutical targets since they show increased expression in several types of tumors. SRPKs have been considered to be constitutively active kinases and the way of their regulation is not completely clarified. A factor reported as determining SRPKs' regulation is their sub-cellular partitioning. Although the most prominent functions of SR protein kinases are nuclear, their sub-cellular localization is primarily cytoplasmic. The aim of this work was to study the intracellular localization of SRPK1 under metabolic stimuli, such as glucose and amino acid deprivation, oxidative and osmotic stress, hypoxia and others. To this end, HeLa and MCF-7 cells were cultured under the above mentioned conditions or their combinations and we show their effect on SRPK1 protein levels and intracellular localization by biochemical fractionation, western blotting and immunofluorescence.

P199 Intranuclear localization of core pluripotency factors' loci in murine ES cells

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It is already known that mammalian nucleus is structurally and functionally compartmentalized. Analysis of the location of genes and chromosomes in a number of cell types has revealed that genomic elements occupy preferential positions within the nucleus. Repositioning occurs during many physiological processes and positioning patterns are evolutionary conserved pointing to a functional role in genome activity. The strongest support of the relevance between intranuclear site and gene activity comes from the observation of movement of several genes from a peripheral position into the interior upon their activation. Most of these genes are associated with the differentiation process so we found intriguing to investigate if this happens also in case of the core pluripotency factors Nanog, Oct4 and Sox2 during the differentiation of murine Embryonic Stem cells. These genes are active in stem cells and silenced upon cellular differentiation. Their intranuclear loci have so far been studied only in humans and with respect to their Chromosome Territory. We performed three-dimensional DNA FISH analysis of the radial position of Nanog, Oct4 and Sox2 loci on three differentiation stages: undifferentiated ES cell, Embryoid Bodies and Neural Progenitor cells. So far we have found that these genes are located close to the nuclear periphery independent of their activity status, although in mammalian systems this region is regarded as a repressive compartment. Interestingly, in the case of Oct4 locus we observed a further movement towards the periphery, when this gene is silenced.

P200 REGULATION OF COUP-TF IN THE SEA URCHIN Paracentrotus lividus

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Coup-TF is a highly conserved gene in all metazoans, from Hydra to humans, which plays an important role in organogenesis and neurogenesis. Recent mutagenesis studies from our laboratory indicate that an upstream 320bp module is responsible for its specific late embryonic regulation in the oral ectoderm. Specifically, three response elements within this module, located at positions -453, -432 and -377, seem to be necessary and sufficient for correct quantitative and spatial regulation. Thus, mutation of the -453 element results in reduced oral ectoderm expression at pluteus stage, whereas mutations at -432 and -377 sites result in aberrant expression in additional embryonic territories. Therefore, the transcription factor(s) that binds the -453 element acts as a positive regulator in contrast with the factors that bind to -432 and -377 elements, which act as negative regulators. Following an *in silico* analysis of the three binding sites, we cloned cDNAs for the transcription factors PlElk (a member of the Ets family) and PlOtx (orthodenticle related homeodomain), considering them candidate proteins that bind the -453 and -377 sites respectively. Using RT-PCR and *in situ* hybridization we demonstrated that the developmental expression pattern of the two genes is similar to Coup-TF's expression pattern at all embryonic stages. *In vitro* translated PlElk and PlOtx specifically bind the corresponding -453 and -377 elements, as shown by EMSA experiments. The *in vivo* regulatory role of PlElk and PlOtx, is currently studied by microinjection of morpholino antisense oligonucleotides. The factor that binds at position -432 is still elusive.

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SOS regulation in the bioethanol-producer Zymomonas mobilis

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Zymomonas mobilis is an α-proteobacterium considered for industrial bioethanol production. Its tolerance to mutagenic stress is of major interest in both basic and applied directions. Genes induced by mutagenic stress in prototypical organisms such as *E.coli* include DNA-repair genes and are coordinately regulated by the RecA/LexA proteins, comprising the SOS regulon. In this regulon, LexA is the global gene repressor and binds to operators called SOS-boxes; upon DNA-damage induction, it undergoes autocleavage with the aid of RecA and allows for gene de-repression. In this work, we studied the presence and constituents of an operating SOS system in *Z.mobilis*, via genetic and computational approaches. We found that SOS regulation highly likely exists in *Z.mobilis*, since (i) the predicted RecA and LexA orthologs bear domain conservation relevant to those of *E.coli*, (ii) the *Z. mobilis recA* gene is inducible under DNA-damage conditions and (iii) the *Z.mobilis recA* gene complements an *E. coli recA*$^-$ host. Computational whole-genome searches for SOS-box presence prior to genes of different *Z.mobilis* strains, revealed perfect or near-perfect SOS-boxes preceding DNA-repair genes and an impressively large number of genes involved in metabolism, protein synthesis, tolerance to inhibitors and transcriptional regulation. This analysis also led to the determination of the consensus SOS-box motif for *Z.mobilis*. Lastly, a *recA*$^-$ *Z.mobilis* strain (UA1) was constructed via allele exchange and its analysis corroborated the *recA*$^-$ recessive phenotype: susceptibility to mutagens and recombination deficiency. Interestingly, though, UA1 proved to be a higher ethanol-producer compared to the parental strain, which invites for further studies.

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The photoperiodic counter in the moth Sesamia nonagrioides

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Various organisms, including insects, have acquired the ability to measure the ratio of day and night (photoperiod) in order to distinguish the long summer days from the short winter ones. Responses to photoperiod (photoperiodism), provide organisms with valuable information regarding the calendar time (season), which enables them to predict the arrival of harsh seasons and adjust their lifestyles and developmental destiny to thrive. Photoperiodism induces the phenomena of diapause and seasonal morph determination at different ontogenetic stages of insect species. However, the functional molecular elements involved in the photoperiodic response are still veiled. In this study, we examined the role of the circadian clock in the regulation of diapause in the insect *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *S. nonagrioides* has numerous advantages in order to investigate diapause, such as larval facultative diapause and photoperiodic control of the induction and the termination of diapause. Previously we cloned ***Cyc, Cry, Per, Tim***, *Clock*, in the corn *S. nonagrioides*. Here we examined by Real Time RT-PCR analysis, the expression of *SnoPer* in the head of larvae, reared under L16:D8, L10:D14, L10:D62 and L10:D14:L10:62D conditions. Under 16L:8D conditions, the *SnoPe*r mRNA levels peaked 5h after the beginning of the scotophase; under 10L:14D, the *SnoPer* mRNA levels peaked at the starting of the scotophase; under L10:D62 the *SnoPer* mRNA levels peaked at the end of the scotophase, and finally, under L10:D14:L10:62D the *SnoPer* mRNA levels peaked again, at the end of the scotophase. These results indicate the circadian system as a part of the photoperiodic system.

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Tip60 protein is a transcriptional activator of the interleukin-2 (IL-2) gene and the HIV-1 virus

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Tip60 is an HIV-1-Tat interacting acetyltransferase which participates in the transcriptional repression and activation of several mammalian genes. In the current study we investigated the tip60 gene expression profile in naive T helper $CD3^+CD4^+CD45RA^+$ (Th) lymphocytes isolated from cord blood, $CD3^+CD4^+CD45RO^+$ memory Th lymphocytes isolated from peripheral blood and the T-cell leukemia Jurkat. We determined the effect of tip60 on the transcription of IL-2 and HIV-1 by co-transfection experiments. Determination of tip60 mRNA levels by qRT-PCR revealed that tip60 is expressed in higher levels in resting memory compared to resting naive Th cells. Tip60 is also expressed in Jurkat cells in levels similar to naïve Th lymphocytes. Activation of the cells with the mitogens PMA/IONO led to a further increase of tip60 mRNA synthesis in memory Th cells, but not in naïve Th or Jurkat cells. That was not observed for naïve Th lymphocytes and Jurkat cells. The same pattern of tip60 expression was observed at protein level. Transfection experiments in Jurkat cells with the plasmid PCMX-tip60 that overexpresses Tip60 led to the induction of IL-2 as showed by qRT-PCR. Moreover co-transfection experiments in Jurkat cells with increasing amounts of PCMX-tip60, led to a gradual increase of HIV1-LTR-CAT transcriptional activity. Our data demonstrate that Tip60 is expressed differentially in naïve and memory helper T cells and participates in the transcriptional activation of both HIV-1 LTR and IL-2. The transcriptional activation of IL-2 by Tip60 protein may account for the induction of pathological disorders of the immune system (i.e. autoimmune diseases).