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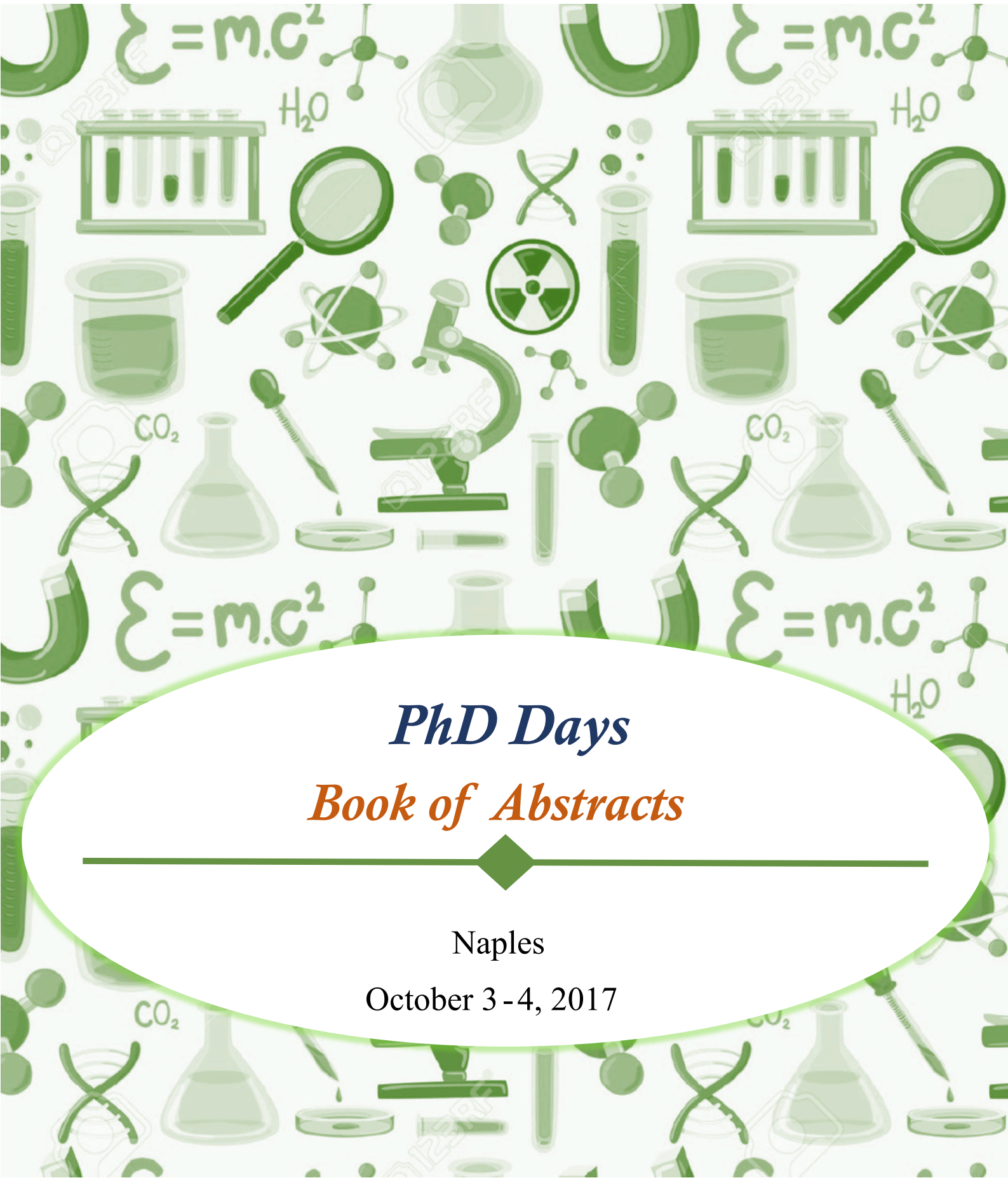

PhD Days

Book of Abstracts

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Session 1 :

Molecular Cell Biology

Mechanism of Interaction of Glycerophosphoinositol and Shp-1

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Glycerophosphoinositols are biologically active metabolites produced from membrane phosphoinositides by phospholipase A_2IValpha. When added exogenously, the glycerophosphoinositols can enter cells and have multiple effects. Previous studies conducted in melanoma cells have unveiled that glycerophosphoinositols impair tumor cell migration through the extracellular matrix. Additionally, glycerophosphoinositols act as paracrine factors in a negative feed-back loop that decreases pro-inflammatory response in LPS-treated human monocytes affecting the expression of key pro-inflammatory mediators. With the aim to elucidate the underlying mechanism of action of glycerophosphoinositols our lab has found that the tyrosine phosphatase Shp1 acts as a specific intracellular receptor of the glycerophosphoinositols. Preliminary data from our lab has shown that it is involved in both cancer progression and inflammation, where glycerophosphoinositols are active. In order to ascertain the domain involved in the regulation of the phosphatase activity and in the binding of glycerophosphoinositols to Shp1, we are performing NMR spectroscopy, mutagenesis and structural analysis studies which have so far led to the identification of specific Shp1 residues involved in the glycerophosphoinositol binding and activities. The full definition of the specific protein domain/s involved in the binding of the glycerophosphoinositols will allow the full definition of the mechanism of action of these compounds.

Novel fluorescent probes for precision labeling in super-resolution microscopy

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PhD cycle**:** 32° cycle.

The development of novel bioconjugation strategies is becoming a very broad field of research,which has a strong impact on many fields related to life science, such as biomedicine, theranostics and advanced imaging. My project is focused on the development of novel staining reagents for super resolution imaging. Currently, a major bottle-neck in super resolution imaging systems is related to the precision of labeling of the protein target of interest. For instance, normal indirect immunofluorescence techniques place the fluorophore at a distance of 20 nm away from the target, essentially preventing full exploitation of super resolution power of modern microscopes. We have developed a Fab-based technology to place fluorescent tags at nearly zero-distance from the protein of interest. Among methods for antibody labeling, the use of amino-reactive fluorophores allows the preferential linkage to primary amino groups. All Fabs contain primary amino groups; α-amino groups situated in the N-terminal amino acid and the ε-amino group of lysine residues.We have set up an N-terminal selective reaction without involving the lysine amino-group by a pH reaction control. N-terminal amino groups are situated very close to the epitope recognition surface, so tags located there are very close to the epitope. The aim of my research project is the characterization of the selective N-terminal labeling reaction of the Fab, in order to develop a general method for the characterization of fluorescently labeled products and then optimize reaction conditions. The ultimate goal is the production of powerful and novel tools for super resolution imaging in fixed and intact cells.

Unravelling the molecular details of AUTS2 regulation by Gb3.

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Glycosphingolipids(GSLs) are a heterogenous group of amphipathic lipids composed of a hydrophobic ceramide base and a sugar headgroup. The ceramide is integrated in the cellular membranes whereas the sugar headgroup is mostly seen facing the non-cytosolic space. GSL synthesis begins in the endoplasmic reticulum where ceramide is produced either by *de novo* synthesis (condensation of a sphingoid base with acyl-CoA) or via the salvage pathway (lysosomal degradation of GSLs). The ceramide is then transported to the Golgi where it is glycosylated by distinct GSL synthesizing enzymes (GSEs) and transported to the plasma membrane. GSLs have been shown to act as first and second messengers in signal transduction and molecular recognition processes via lateral interaction with surface receptors. Data from our lab suggested that there exists a novel mechanism whereby Gb3 (goloboside) negatively regulates the expression of the epigenetic modulator Autism Susceptibility Candidate 2 (AUTS2). AUTS2, in turn, binds GM3S promoter inducing local histone hyper-acetylation resulting in GM3S expression and other AUTS2 regulated genes. Currently, we are characterizing the molecular signalling events which regulate this process and thereby uncovering the role of GSLs in signal mediated cellular response in key biological events like differentiation. We will present the preliminary data on signalling events which control this internal regulation of GSLs, mediated by *NF-κB* pathway.

Effects of T3 and 3,5 T2 on insulin sensitivity and related metabolic changes in muscle cells treated with fatty acids

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T3 and its metabolite 3,5-T2 both increase skeletal muscle insulin sensitivity but it is unknown if they act directly on muscle or only indirectly through increased hepatic FA oxidation. Furthermore, the metabolic effects of thyroid hormones in animal models vary with the diet's FA composition. To investigate this, we studied whether T3 and 3,5-T2 prevent insulin resistance in rat myotubes induced by FAs with different saturation degrees.
In the presence of insulin, the reduction of Akt phosphorylation in response to palmitate was fully prevented by 3,5-T2 (confirming data obtained in skeletal muscle of rats treated with diets based on saturated FAs), and only partially by T3. Oleate- mediated enhanced insulin sensitivity was normalized by 3,5-T2 and T3, instead linoleate-mediated enhanced insulin sensitivity was further enhanced by 3,5-T2 and T3. Cellular respiration was reduced by palmitate and normalized only by T3, through ATP synthesis-independent respiration. PGC-1alpha expression reflected insulin-induced Akt phosphorylation caused by the FAs and thyroid hormones.
These results show that 3,5-T2 and T3 can act directly on muscle cells by differentially modulating FA- induced insulin resistance, which indicates that dietary backgrounds profoundly alter the effects of thyroid hormones on muscle insulin sensitivity in vivo.

The IL8-CXCR1/CXCR2 circuit in Thyroid Cancer

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Interleukin 8 (IL8/CXCL8) is a crucial paracrine/autocrine factor that induces the epithelial-to-mesenchymal transition (EMT) and increases stemness features of thyroid cancer (TC) cells by binding CXCR1 and CXCR2 receptors. Here, we aim to dissect the molecular mechanisms involved in IL8-mediated biological activity in TC and to assess the impact of such mechanisms on TC cell tumorigenicity/metastatic phenotype. To this aim, RNA sequencing was performed on IL8 treated and not treated TC cells (8505c). Analysis of the obtained data identified 571 over-expressed and 264 down regulated genes upon IL8 treatment of TC cells. Further analysis of RNA seq data was performed for gene set enrichment analysis of Hallmarks of cancer. KRAS, UV Response, Hedgehog Signaling, Apical Junction, Mitotic Spindle, Wnt/Beta-Catenin, Epithelial Mesenchymal Transition, TGF-Beta, Notch, Hypoxia, Apical Surface, Complement and Heme Metabolism signaling pathways were positively enriched and Oxidative Phosphorylation (OXPHOS) pathway was significantly negatively enriched by IL8. Transcriptional targets of NANOG, SOX2 and OCT4 were identified from the NOTCH, Hedgehog, Wnt/Beta-Catenin Signaling and OXPHOS pathways, that were significantly changed in their expression after IL8 treatment. These changes in gene expression were further validated in TC patients genomics data sets retrieved from cBioPortal. NEAT1, a long non-coding RNA and a positive regulator of IL8 expression was the most up-regulated target upon IL8 treatment. After validation of these results in a panel of TC cell lines treated or not with IL8, by qPCR and western blot, functional assays will be performed.

The Lysophosphatidic Acid Acyltransferase (LPAATs) Enzymes and their Role in Membrane Transport Alterations in Cancer

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The 1-Acyl-Glycerol-3-Phosphate AcylTransferase enzymes (AGPATs) is an emerging class of enzymes, and the AGPAT2 member is a potential prognostic/diagnostic marker for different tumors. AGPAT2 is upregulated in several tumors and its increased expression/activity correlates with tumor aggressiveness. AGPAT2, also known as LysoPhosphatidic Acid AcylTransferase-β (LPAATβ), catalyses the acylation of lysophosphatidic acid to form phosphatidic acid, a phospholipid precursor involved in membrane transport and signalling for cell survival, proliferation and tumor progression[1]. Alteration in phospholipid membrane composition and enhanced cellular secretion are associated to tumor progression and migration/invasion. Indeed, during tumorigenesis the secreted factors of cancer cells are key actors in setting the microenvironment that leads to tumor progression.

In addition to AGPAT2, also AGPAT3, 4, 8 and 11 are up-regulated in cancer cells. AGPAT4, like AGPAT2, is a LPAAT enzyme, and we have identified AGPAT4 as an important controller of secretion in cancer cells[2]. AGPAT4 expression/activity increases in prostate cancer, and this correlates with enhanced tumor aggressiveness. Conversely, its depletion impairs cells migration. However, the role of AGPATs in the exocytic pathways that drive tumor progression and migration/invasion remains unknown.

We performed a proteomic approach to identify, in a sensitive, and at high-resolution manner, factors differentially secreted in the conditioned medium of prostate cancer *versus* non-cancer cells, by LC-MS/MS. Then, we have used the same differential proteomic approach to identify among these cancer-specific secreted factors those with reduced secretion under AGPAT4 depletion. Among them, we have found the human Growth Hormone and the membrane type 1- and type 9-matrix metalloproteases[3], which are all involved in invasion/migration.

The role of the identified AGPAT4-dependent cancer-secreted factors in tumor invasion/migration, are under investigation.

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Obesity-Driven Neurodegenerative diseases: New insights for new molecular interplayers and therapeutic targets

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The neurodegenerative diseases are one of the most relevant causes of worldwide disability and are increasingly linked with obesity. Neurodegenerative and dysmetabolic diseases share some molecular pathways in many cerebral areas starting from the hypothalamic one. In this area we studied the molecular pathway, which controls the balance between the not phosphorylated and phosphorylated form of Tau, the protein responsible for maintaining the axonal stability. We found that different molecular interplayers as leptin, orexin-A (OX-A) and the endocannabinoid system are altered in obese and neurodegenerative phenotypes by changing the GSK-3β activity responsible for the phosphorylation of Tau which is implicated in the synaptic plasticity.
Our findings show that leptin deficiency leads to an increase of OX-A-induced biosynthesis of the endocannabinoid 2-arachidonoylglycerol (2-AG) paired with a pronounced increase of pTau/Tau ratio, this condition was reverted after leptin treatment. On this basis, we hypothesized a functional orexin/endocannabinoid/leptin interaction as an upstream signaling pathway for the regulation of Tau phosphorylation in the hypothalamus. Unraveling the functional cross-talk between ECs and OX system in the regulation of Tau phosphorylation also in hippocampus, the main brain area involved in cognitive function could reveal novel molecular targets as a possible therapeutic approach.

Synergic effect of curcumin and ellagic acid on genomic stability of human amniotic cells

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Pregnancy is susceptible to oxidative stress closely related to the pathogenesis of many fetal diseases. It is known that many natural antioxidant molecules are able to counteract oxidative imbalance, so this study has been focused on evaluating the individual antigenotoxic effects of ellagic acid and its combination with the curcumine on human amniotic cells in vitro. We presents new data on the DNA damage in amniotic cells exposed in vitro to a known oxidant agent H_2O_2 (15 μM) and to the curcumine (40 μM) plus ellagic acid (100 μM) for different times (48 and 72 hours). The genotoxicity has been highlighted by using two different experimental approaches (TUNEL test and RAPD-PCR technique).The results of the TUNEL test showed a statistically significant increase of the DNA fragmentation after 48 hours of exposure. The co-exposure to the antioxidants shows DFI% values comparable to that of the negative controls already starting from the minimum exposure time. The RAPD- PCR analysis showed a variation of the polymorphic profiles of the amniotic cells DNA exposed to H2O2 with respect to the control amniotic cells DNA. The evidence from the value of GTS showed statistically significant increase of the damage to DNA caused by H_2O_2, as opposed to an increase in the genomic stability of the template in the samples treated with the combination of the two molecules.

Secretory phospholipase A_2 role in osteoclastogenesis

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Reported evidence indicate the involvement of the secretory phosholipase A_2 group IIA ($sPLA_2$-IIA) in osteoclastogenesis, a process leading to the formation of multinucleated professional bone-resorbing osteoclasts.
To elucidate the role of this glycerophospholipid-hydrolytic enzyme, the macrophagic Raw264.7 cell line was chosen as osteoclast precursors, which differentiate during 5-7 days of treatment with Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL).
Since $sPLA_2$-IIA is a dual functional protein, to dissect its mechanism of action, inhibitors with distinct selectivity against $sPLA_2$-IIA actions were used. A highly hydrophobic pentapeptide [20 mM, c(2NapA)LS(2NapA)R] and a cell-permeable small molecule (40 mM, KH064), both able to block $sPLA_2$-IIA catalytic activity together with its binding to other proteins, decreased the transcription of osteoclast markers and the multinucleated-cell formation during RANKL-induced osteoclastogenesis. The down-regulation of $sPLA_2$-IIA expression, by small-interfering-RNAs in precursor cells, further confirmed these data. Instead, the treatment with the cell-impermeable bifunctional inhibitor LY311727 (30 mM) or with an inhibitor of $sPLA_2$-IIA catalytic activity (10 nM p-bromophenacyl bromide) reduced osteoclast maturation without blocking syncytium formation.
These results indicate that $sPLA_2$-IIA participates in osteoclast maturation in a catalytically-dependent manner, while its control of syncytium formation is catalytically-independent and is probably due to $sPLA_2$-IIA interaction with an unidentified partner in an intracellular compartment.
Notably, c(2NapA)LS(2NapA)R and KH064 treatments regulated RANKL-induced signalling, decreasing p38 activation, which can depend on $sPLA_2$-IIA interactions with membrane receptors. Moreover, a p38 inhibitor (25 mM SB203580) impairs the multinucleated-cell formation without affecting osteoclast maturation, indicating the involvement of p38 signalling downstream $sPLA_2$-IIA in osteoclast fusion.

Unraveling the inflammatory cells contribution of Cripto to skeletal muscle regeneration

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Regeneration of skeletal muscle tissue is a highly coordinated cascade of events, including the interactions of different cell types, such as muscle stem cells and inflammatory cells. Inflammation has beneficial effects on skeletal muscle regeneration and is mainly driven by two types of macrophages: pro-inflammatory (M1) and anti-inflammatory (M2). More specifically, M2 macrophages are involved in tissue repair processes such as matrix deposition and angiogenesis. Our recent data point to a key role that Cripto plays in this context. Cripto is a developmental growth factor and, after muscle injury, is re-expressed both in activated satellite cells and in a subset of macrophages. To date the role of Cripto in myogenic compartment has been partially elucidated, however, its role in inflammatory cells remains elusive. Thus, we generated two mouse models to abrogate Cripto expression in macrophages: a model based on the transplant of Cripto-KO haematopoietic progenitors and a myeloid lineage-specific Cripto loss-of-function genetic mouse model. After muscle acute injury, both models lacking Cripto show an impairment of M2 macrophages accumulation. Furthermore, according to decrease of M2 macrophages, we observed defective angiogenesis during a time course of skeletal muscle regeneration. Our findings suggest a novel role for Cripto in the network by which macrophage-mediated inflammatory response promotes vascular and muscle repair.

The Ultraconserved Long Noncoding RNA, T-UCstem1, is required to preserve transcriptional identity and maintain Embryonic Stem Cell self-renewal"

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Ultraconserved elements (UCEs) show the peculiar feature to retain extended perfect sequence identity between human, mouse, and rat genomes. Most of them are also transcribed and identify a new family of lncRNAs, the transcribed UCEs (T-UCEs), but their function is largely unknown particularly in stem cells. We focused on uc.170, named T-UCstem1, and we highlighted a functional interplay between it and the neurogenic miR-9. Such molecular interplay is crucial in preserving the ESC proliferation; indeed, T-UCstem1 Knock-down embryonic stem cells ((T-UCstem1 KD ESCs) showed an increased mir-9 levels leading to a cell cycle perturbation. Further analysis showed that a T-UCstem1/miR-9/Tlx1-Lin28b axis controls cell cycle progression in ESCs. Specifically, by using antagomiR-9, we showed how in T-UCstem1 KD cells the reduction of mir-9 extralevel led to the miR-9 target genes (Tlx1 and Lin28) expression restore, by rescuing T-UCstem1 KD ESC proliferation.
By further analysing the molecular and cellular features of T-UCstem1 KD cells, we showed that, despite their altered proliferation rate, T-UCstem1 KD ESCs retained a proper pluripotency *in vitro* and *in vivo*. Additionally, we compared RNA-seq profiling of T-UCstem1 KD and Control ESCs. The analysis showed a large number of the gene up-regulated in T-UCstem1 KD ESCs and most of them are bivalent domains-associated genes. Such observation led us to hypothesize a possible involvement of T-UCstem1 in preserving the epigenetic status of such regulative elements, possibly trough Polycomb Repressive Complex 2.

Isolation and functional characterization of human miR-125a promoter”

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MicroRNA-125a-5p (miR-125a) is the vertebrate homolog of lin-4, the first discovered microRNA, and plays a fundamental role in embryo development by downregulating Lin-28 protein. MiR-125a is also expressed in differentiated cells where it generally acts as an antiproliferative factor by targeting membrane receptors or intracellular transductors of mitogenic signals. MiR-125a expression is downregulated in several tumors, including hepatocellular carcinoma (HCC) where its targets sirtuin-7, matrix metalloproteinase-11, VEGF-A, Zbtb7a, and c-Raf. In this study, we have isolated the transcription promoter of human miR-125a and characterized its activity in HCC cells. It is a TATA-less Pol II promoter provided with an initiator element (INR) and a downstream promoter element, located 3939bp upstream the genomic sequence of the miRNA. The activity of the promoter is increased by the transcription factor NF-kB, a master regulator of inflammatory response, and miR-125a itself was found to strengthen this activation through inhibition of TNFAIP3, a negative regulator of NF-kB. This finding contributes to explain the increased levels of miR-125a observed in the liver of patients with chronic hepatitis B. The isolated promoter will be useful for the development of reporter assays to search compounds increasing its activity, eventually leading to treatments to restore miR-125a expression in tumor cells.

Adiponectin expression is specifically related to Common variable immunodeficiency

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Adiponectin is widely studied for its beneficial metabolic properties. It circulates as different oligomers LMW, MMW and HMW. Adiponectin attracted renewed interest since it was associated with the development and progression of immune disorders. The mechanisms underlying this association and the role of Adiponectin in pathophysiology of immune-mediated conditions remain unknown. Common variable immunodeficiency (CVID) is a primary immunodeficiency characterized by impaired antibody production. Recently, we demonstrated that total Adiponectin levels as well as HMW are strongly decreased in CVID patients compared to controls. In this study, to investigate whether Adiponectin concentrations are related to Ig, its levels were analyzed before and after the first Ig replacement therapy in eight CVID naïve and, as control, in five CIDP patients: Adiponectin and HMW levels quickly and dramatically increase after the Ig infusion only in CVID patients. These findings indicate that Ig administration *per se* is not able to increase Adiponectin but the specific cellular and/or molecular background proper of the CVID disease seems to be essential. In conclusion, our data demonstrated that Adiponectin is specifically related to CVID disease activity but further studies are required to understand the biological role of Adiponectin and its possible usefulness as disease biomarker in CVID.

Regulation of PPARg signaling through alternative splicing and dominant negative isoforms

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PPARg is a transcription factor crucial for adipogenesis. Loss-of-function (LOF) mutations in the ligand-binding domain (LBD) originate dominant negative isoforms, associated with T2D and defective neoadipogenesis. PPARg dominant negative isoforms can also generated by alternative splicing, despite their functional significance has not been investigated.
During my PhD I studied a new PPARg isoform, PPARgΔ5, generated by exon 5 skipping. Similar to mutant receptors it lacks LBD, acting as dominant negative.
My results indicate that ligand-mediated PPARg activation is necessary to promote *PPARG* pre-mRNA splicing and that silencing of wild-type *PPARG* or treatment with antagonist does not increase exon 5 skipping. Publicly available CLIP-Seq data revealed that the splicing factor ASF/SF2 binds *PPARG* mRNA, raising the possibility of its contribution to this new PPARg regulatory mechanism. Confirming this hypothesis, its knockdown significantly prevents exon 5 skipping, even in presence of ligand-activated PPARg. RNA-Sequencing in PPARgΔ5 over-expressing cells revealed its dominant activity on whole-transcriptome, with metabolism-related genes being the most affected. Thus, investigating PPARgΔ5 in subcutaneous adipose tissue (SAT) biopsies of obese patients and in adipocyte precursor cells (APCs) isolated from SAT, I found high PPARgΔ5 levels in both the sample types. Because of the crucial PPARg role in energy homeostasis and in adipogenesis, during the last year I'll determine its functional role in a cellular model of human adipogenesis.

3,5-diiodothyronine (T2) targets different pathways to induce white adipose tissue browning

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White adipose tissue (WAT),following appropriate stimuli, acquires a brown fat-like phenotype leading to increased thermogenesis. This phenomenon is known as browning and is more likely to occur in subcutaneous white adipose tissue (SAT) involving many molecular factors, such as several miRNA and the irisin protein. Since it has been shown that a thyroid hormone derivative, 3,5-diiodo-L-thyronine (T_2), is capable to activate BAT thermogenesis, the aim of this study was to verify whether T_2 induce browning and to investigate the underlying mechanism. In SAT of high fat diet (HFD) rats, T_2 administration increases uncoupling protein 1 (UCP1) expression and modulates the expression of miR-133a and miR196a, when compared to normal and HFD animals. Associated to reduction of miR-133a there was an increase of Prdm16 expression, a critical regulator of brown adipocyte development, and associated to an increase of miR-196a there was a reduction of Hoxc8, repressor of adipogenic marker C/EBPβ, which resulted increased. Furthermore, T_2 increased serum levels of the miokineirisin that stimulatesbrowningthrough the ERK pathway. These data demonstrate that T_2 is able to affect important pathways involved in the browning of SAT suggesting another mechanism by which T_2 exerts its stimulatory effect on metabolism.

Insight from *Cripto* gene into neuroectoderm and mesoderm cell lineage segregation

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The relation existing between mesoderm and neuroectoderm during early vertebrate development is highly complex and dynamic. *Cripto* is a key gene for early mesoderm development, whereas its loss of function triggers neural differentiation. The aim of my thesis is to dissect the role of *Cripto* in mesoderm and neuroectoderm cell lineage segregation, by means of gain of function experiments. First of all, I studied the phenotypic effect of *Cripto* overexpression in neural progenitors *ex vivo*, showing a strong inhibition of neural differentiation and suggesting a transdifferentiation towards mesenchymal/mesodermal fate. To confirm these data *in vivo*, I performed *in ovo* transplantation of *Cripto*-neural progenitors in chick embryo epiblast observing a failure of integration in the forming neural tube and, interestingly, their migration into mesenchymal tissue. All these data indicate the high plasticity between neural and mesodermal lineages in the early embryo and demonstrate that *Cripto* alone is able to interfere with the correct neural differentiation and cause an aberrant mesenchymal transformation. Interestingly, I also found a strong enrichment of *Cripto* expression in the mesenchymal subtype of human glioblastoma, suggesting a possible role for *Cripto* in neural tumorigenesis.

In summary, this data point out to a strong role of *Cripto* in regulating neural/mesenchymal differentiation giving new insights into neural development and diseases.

Functional organization of matrix proteins of the Golgi – their contribution to the regulation of glycosylation

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The Golgi matrix proteins (GRASPs and Golgins) contribute to the structural organization of the Golgi apparatus and their role as tethering factors controlling membrane transport at the Golgi apparatus is well established. Studies have also indicated to a role for these proteins in controlling the glycosylation function of the Golgi in an "indirect" manner, through their contribution to the structural organization of the organelle. Here, we have characterized the glycosylation changes associated with the depletion of a subset of matrix proteins localized to the cis/medial Golgi. Our results suggest that the observed changes in glycosylation are matrix protein specific and probably go beyond their contribution to the transport of cargoes at the Golgi. We are presently characterizing the mechanism by which the matrix proteins may organize specific glycosylation reactions at the Golgi apparatus. By using glycosphingolipid biosynthesis as a model system we show that Grasp55, a medial/trans localized protein involved in the stacking of the Golgi cisternae, influences the proper intra-Golgi localization of a key glycosphingolipid biosynthetic enzyme, lactosylceramide synthase (***LCS***), by regulating its sorting into retrograde transport vesicles. Deletion of Grasp55 function leads to a mislocalization of this enzyme, which we show changes the glycosphingolipid output of the Golgi.

Role of PAX8 in the development and differentiation of Oviduct

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PAX8 is a transcription factor of the Paired – box gene family, required for the development of the thyroid, reproductive system, excretory system and brain. PAX8 is observed to be necessary for the development of Fallopian Tube, which has previously not been reported. Our project will aim in understanding the expression pattern and role of PAX8 in the development of Fallopian Tube, composed of secretory and ciliated cells. This will be done by tracing, using immunohistochemistry (IHC) and immunofluorescence (IF), various regions of the Fallopian tube from embryonic stages till the adult stage of female mice. The standardization of IF has been established. The proteins, OVGP1 and Acetylated Tubulin best represent the secretory cells and the ciliated cells of the Fallopian Tube respectively. The role of PAX8 during development can be confirmed by comparing the results of IF and IHC with normal and PAX8 null mice. PAX8 is an important marker of the secretory cells and its role in the development and function of secretory cells is yet unknown. This will be investigated by observing PAX8 knockdown effects on the secretory cells. We can further validate these results on a PAX8 knockdown in Fallopian Tubal secretory cell line and on primary cultures from the Fallopian Tube.

Session 2 :

Gene Regulation and Computational Biology

Research and development of novel algorithms, methods and software tools for the integration and analysis of data produced by high throughput biological experiments

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In first year of my PhD, I am involved in different projects and overall goal of these projects is to devise novel, simpler, more efficient solutions to real-world bioinformatics and computational biology problems in order to provide more insight in biological mechanisms.

First project was the development of tool called "DecontaMiner", which is used for detects contaminating sequences among the reads discarded from the alignment to the reference genome. It provides a pipeline that takes in input the files containing the unmapped reads, allowing a post-alignment investigation that can be integrated to the standard procedures used in NGS data analysis. Our aim is to help researcher in obtaining more information from the data, and, in particular, to check for the presence of microorganisms that not only affect the reliability of the whole experiment, but also foster the evaluation of the samples and the conditions under an additional perspective.

Secondly, We developed a pipeline for comparative analysis of gene expression profiles employing rank based statistical approaches such as Rank-Rank hyper-geometric overlaps (RRHO) and Prototype Rank list (PRL) data analysis methods. It includes four main features: (i) Conversion of expression data into rank matrix, (ii) prototype rank list generation, (iii) Distance calculation from PRL, (iv) RRHO analysis.

In another collaborative project, I am designing a webserver for protein structure validation based on multiple parameters, involved in the variability of protein backbone geometry. These include bond distances, bond angles and dihedral angles. In recent work accepted in Biomed research international, we stated the necessity of considering the variability of protein backbone geometry in structure refinement, validation, and prediction.

Apart from this, I am also working on splicing study of 32 different human tissues using publicly available RNA-Seq data. At present, we have aligned and analysed the gene expression, isoform identification and splicing patterns with in different tissues. In future, we are planning to develop a web based platform for the integration and analysis gene splicing patterns of different tissues with in humans, as well as comparative analyses with other primates. In particular, we are focussing on skeletal tissue muscle, as it is obvious that other primates such as Pan troglodytes have much stronger muscles than it human counterpart. The idea is to study the genetic mechanism behind this phenotypic difference.

Molecular mechanisms for maintenance of genomic imprinting in mouse embryonic stem cells

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In mammals, about a hundred of genes, crucial for development, are monoallelically expressed. The gamete-of-origin-dependent expression, known as genomic imprinting, is controlled by differential DNA methylation, established on Imprinting Control Regions (ICRs) during gametogenesis,.The protein ZFP57, binding the methylated allele at the ICRs, has been shown to be required for imprinting maintenance in early mouse embryo, recruiting the co-repressor KAP1, DNA methyltransferases (DNMT1,3A,3B) and the histone H3 lysine 9 methyltransferase SETDB1. Nuclear Transcription Factor Y (NF-Y) is one of the transcription factors binding the CCAAT box of various gene's promoters. NF-Y's nucleosome-like properties seems to provide a stable binding for pioneer factors Oct4/Sox2 allowing and/or promoting other TFs binding.
It has been reported that NF-Y positively regulates the antisense transcription of Kcnq1ot1, suggesting a crucial role for NF-Y in the organization of the parent-origin-specific chromatin conformation at Kcnq1 ICR. ChIP allele-specific that I performed in hybrid mESC lines, showed that NF-Y binds the Kcnq1 ICR only on the non-imprinted allele. The aim of my project is to investigate what is preventing NF-Y from binding the imprinted allele. Employing mESC lines lacking ZFP57 and SETDB1, we are going to study what is the link between NF-Y transcriptional factor and imprinting maintenance.

Statistical analysis of high dimensional omic data

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The Next Generation Sequencing is a modern technology that allows to investigate several biological questions through a deep sequencing of nucleic acids. Over the last years, large amount of high dimensional omic data have been collected (e.g. scRNA-seq, RNA-seq, ChIP-seq). Nowadays one of the goals of modern biology is to extract information from these datasets by integrating several multi-omic levels. Therefore, the aim of my PhD project is to gain statistical knowledge and biological background in order to develop own statistical framework for data analysis and integration. Based on this target, my first year activity was mainly focused on RNA-seq data exploration. Specifically, I analyzed transcriptomic data from patients affected by intellectual disability and healthy controls in order to investigate deregulated transcripts between the two conditions. The results of downstream analysis were further processed by our collaborators to carry out a practical investigation in a biological context. My further scientific work was devoted to single-cell RNA-seq study. In June I have visited University of Sheffield in order to establish scientific project collaboration on scRNA-seq data modelling. Together with our collaborator we are currently developing statistical models that will be implemented and used for real data analysis. As for the future work, the above mentioned research activities will consolidate knowledge about novel machine learning methods with focus on the relevant methodology to multi-omic data integration.

Transcriptional and epigenetic deregulation of glycosphingolipid metabolism in Rett syndrome models

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Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked gene Methyl-CpG-binding Protein 2 (MECP2), encoding an epigenetic modulator of transcription. To date, the pathogenetic mechanism of RTT is still unclear.
Correlations between RTT and glycosphingolipid (GSL) metabolism are emerging. Indeed, GLS abnormalities were found in brain of RTT patients and mutations in ST3GAL5 gene, encoding a key enzyme for ganglioside biosynthesis, have been found in patients with a RTT-like phenotype.
Interestingly, AUTS2, whose mutations are associated with autism spectrum disorders, is coherently altered in brain of mice lacking or overexpressing MeCP2. AUTS2 promotes the expression of neuronal genes and regulates neuronal differentiation by influencing GSL biosynthetic pathway.
Our data suggest that MeCP2 may influence GSL metabolism. Indeed, in mouse brain MeCP2 regulates the expression of Auts2, and both proteins bind the promoter of ST3GAL5, with a putative regulatory role. AUTS2, in turn, binds Mecp2 promoter. Furthermore, globosides, GSLs poorly represented in neurons, negatively regulate MeCP2 and AUTS2 expression during neural differentiation.
To decipher molecular mechanisms that links MeCP2, AUTS2 and GSL deregulation, we are currently setting a genome-wide approach, that includes RNA-sequencing and ChIP-sequencing, taking advantage of cellular and murine RTT-models.

Development of novel approaches and pipelines for the integration and analysis of high throughput transcriptomics data.

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I am working in a capacity of research fellow (Bioinformatician) at Institute for High-Performance Computing and Networking (ICAR), National Research Council, Naples, Italy, and also enrolled as a 2nd year PhD student in Molecular Biology, at Universita degli Studi della Campania Luigi Vanvitelli, Naples. During second year of my PhD research, I worked on several interesting projects related to the integration and analysis of high throughput transcriptomics data. Some of these projects are as follows: i) Development of a computational integrative approach based on alternative splicing analysis to compare immortalized and primary cancer cells. Here with my collogues, we introduce a novel strategy based on alternative splicing detection and integration of DNA and RNA sequencing data, to explore the differences between immortalized and tissue-derived cells at isoforms level. Furthermore, in order to better investigate the heterogeneity of both cell populations, we took advantage of a public available dataset obtained with a new simultaneous omics single cell sequencing methodology. The proposed pipeline allowed us to identify, through a computational and prediction approach, putative mutated and alternative spliced transcripts responsible for the dissimilarity between immortalized and primary hepato carcinoma cells, manuscript based on this work is published in Special Issue of The International Journal of Biochemistry & Cell Biology 2017; ii) Development of a mixed integer programming-based global optimization framework for analyzing gene expression data to characterize sub-types of breast cancer in collaboration with Dr. Giovanni Felici, IASI-CNR. The manuscript is published in J. Glob. Optim. (2017); iii) Being a member of LAB-GTP team along with my supervisor Mario Rosario Guarracino and Maurizio Giordano, I also took part in SBV-IMPROVER systox challenge organized by Phillip Morris international, Neuchâtel, Switzerland 2016, to verify that robust and sparse human-specific or species-independent gene signatures predictive of smoking exposure or cessation status, can be extracted from whole blood gene expression data from human, or human and rodents. We won the best performers award for developing one of the best methods and wrote up part of manuscript published in Computational Toxicology, Elsevier 2017.

Apart from this, in 2017, I also work as a leading author on the development of novel computational tool to integrate and analyze functional genomics data such as *Ranker: a graphical user interface for comparing expression profiles using rank based statistical approach.* Results are going to be submitted in the journal paper. In this project, we carry out a comparative analysis of gene expression profiles, and develop a web-based interface "Ranker" employing Rank-Rank hyper-geometric overlaps (RRHO) and Prototype Rank List (PRL) data analysis methods. It includes four main features: (i) Conversion of expression data into rank matrix, (ii) prototype rank list generation, (iii) Distance calculation from PRL, (iv) RRHO analysis. Front-end interface of the Ranker is developed using in-house PHP, Java Scripts and HTML scripts. Core scripts for PRL based distance calculation and RRHO analysis is written in R and "Gene Expression Signature" and "RRHO" packages from Bioconductor are utilized. We presented this work in BMTL 2017 conference in

Naples. In collaboration with my peers, I substantially worked to analyze gene perturbation expression profiles, to reconstruct a directed gene interaction network and decipher the regulatory interactions among genes involved in protein transport signaling. Network biology approach is implemented to delineate the gene-centric cross-talks at the level of specific modules corresponding to a different signaling pathways. The work is presented in BMTL 2017, Naples and presently we are writing a journal paper based on this work.

Presently, I am working on another computational pipeline for the identification of Circular RNA's from neocortex developing time series high throughput sequencing data. The goal is to determine the non coding RNA (specifically Circular RNA) expression and subsequently elucidate the functional importance of circular RNA's with respect to miRNA regulation.

Changes in the methylation state of DDO, DAO, DAOA and SR genes as a putative mechanism associated to their expression levels in mammalian brains

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The flavoenzyme D-aspartate oxidase (DDO) catalyzes the degradation of the NMDA receptor agonist, D-aspartate, a D-amino acid enriched in the embryonic mammalian brain. DDO is selectively expressed during postnatal life, thus keeping D-aspartate at low levels throughout adulthood. In the first year, we demonstrated that the postnatal expression of *Ddo* is critically regulated by methylation changes within *Ddo* promoter. During the second year, we evaluated whether this epigenetic mechanism predicts not only temporal, but also regional variations in *DDO* expression. To this aim, we analyzed *DDO* methylation and expression in hippocampus, cortex and cerebellum of adult mice and humans. We extended our analysis to the genes involved in the metabolism of the NMDAR co-agonist, D-serine: *D-amino acid oxidase* (*DAO*), *PLG72* and *Serine racemase* (*SR*). Differently to the developmental regulation of *Ddo*, we found no clear association between regional changes in gene methylation and expression in the three brain areas of both mice and humans. The same observation applies to *SR*. On the other hand, *DAO* methylation state could be associated to its differential regional expression, in mice but not in humans. Finally, the primate-specific gene, *PLG72*, shows an overall strong methylation percentage, which is in line with undetectable mRNA levels.

SecreTool: SECREtory Signaling Across Secretory Pathway TOOL

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The secretory pathway is characterized by a series of steps that the cell uses to move proteins inside and outside. In order to maintain the homeostasis, these membrane fluxes need to be balanced and regulated. In literature, there are some evidence of this regulation: it is reported a Cis-Golgi based signaling circuit in which the KDEL receptor acts like a sensor of traffic activating a Src-PKA machinery that promote the acceleration of retrograde and anterograde traffic. According to this, the secretory pathway might be regulated by a series of signaling and transcriptional circuits that have the aim to stabilize a harmonic condition of homeostasis in the cell known as "control systems points". These cascades have the form of usual signaling pathways but, instead of changing the functional state of the cell in response to external stimuli, they have the aim to control the internal state of the cell, responding to internal perturbation and reestablishing the cellular equilibrium. Our idea was to reconstruct signaling pathways within secretion compartments, in order to follow the signaling cascades through organelles and so build up a tool with the capability to reconstruct and compartmentalize signaling pathways within cell organelles. We downloaded information on protein location for signaling proteins from Human Protein Atlas and searched physical and functional interactions on STRING online available tool, in order to represent them in form of 2D network and 3D multilayer network having a picture of pathway cascades thought cell organelles. For now, the tool was tested on pathways well known and then it will be applied on the total amount of signaling proteins

Optimization of variant calling in problematic cases and its application to solve biological questions

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The genomic variation is expressed in several forms which differ for the length of the variation, the most abundant among them is the single nucleotide polimorphism (SNPs). Understanding the distribution of genomic variation is the basis of population genetics, however deriving data for SNPs is not always straightforward either because the source of the data is hard to deal with or because the design of the experiment is not enough informative.
During my PhD, I have optimized two pipelines for SNP calling from ancient DNA and pooled RNA sequencing data, respectively from DNA of human and form the non-model organism *P. perniciosus.* Once I obtained reliable call sets I applied basic population genetic analyses to both cases.
For the DNA-sequencing data I analyzed modern and ancient human DNA data to investigate a signal of positive selection at the SNP rs10180970. This variant is located in the *ABCA12* gene that code a protein implicated in lipid transportation in the skin. There is an unusual unbalance of allele frequencies at rs10180970 between African and non-African populations. With my work I reconstructed the pattern in geographical space and time since neolithic of the rs10180970 alleles and made basic functional characterization of the two alleles in cell cultures.
For the pooled RNA-sequencing data I analyzed a laboratory population of *P. perniciosus* to investigate to which extent genetic variation was reduced and find significant differences between males and female genes. Before being applied, the pipeline for SNP calling in *P. perniciosus* was first optimized with human data. In humans both whole genome high quality DNA sequencing and reference sequence were available and the RNA sequence based variant calls were compared with the corresponding DNA sequence based variants to assess the goodness of the method.
Altogether my work demonstrates the importance of assessing genetic variation prior to population genetic analyses and in both cases it helped clarify biological questions related to genomic variation.

“PRDM molecular variants *PR-plus* and *PR-minus*: role in physiological differentiation and neoplasia”

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PRDM proteins share a PR (Positive Regulatory) domain followed by various Zn-finger repeats. They are involved in proliferation and differentiation control and notoriously act as oncosuppressors. Common feature of PRDM genes is to express two molecular variants (*PR-plus* and *PR-minus*) generated by alternative splicing or alternative use of different promoters. The imbalance between these two forms in favour of the *PR-minus* (yin-yang) characterizes many human tumors.
The relationship between PRDM genes and lymphocytic regulation led to choose lymphocytes as physiological model for studying the expression profile of this gene family. The obtained results allowed selecting the most interesting genes to be analysed after lymphocytic activation and MAPK/NF-kB signalling inhibition. These data let hypothesize a PRDM2 role in the activation of T lymphocytes. In order to determine the cause/effect link of changes in PRDM2 variants expression and their influence on other PRDM genes (e.g. PRDM1/Blimp1), experiments of cell transfection, both in transient and stable were performed. This study aims also to shed light on the mechanism in the tumor-promoting function of the *PR-minus* form, which is still unclear.
Finally, a comprehensive pan-cancer analysis is ongoing to obtain a complete picture of the genomic and transcriptomic alterations for all PRDM genes across human cancers.

Inferring cross-talk among genes within the signaling pathways in intra-cellular protein transport: an integrated approach

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The endomembrane system, known as secretory pathway, is responsible for the synthesis and transport of protein molecules in cells. Therefore, genes involved in the secretory pathway are essential for the cellular development and functions. Recent scientific investigations show that Endoplasmic Reticulum (ER) and Golgi apparatus may provide a convenient drug target for cancer therapy. On the other hand, it is known that abundantly expressed genes in different cellular organelles share interconnected pathways and co-regulate each other activities. The cross-talk among these genes, plays an important role in signaling pathways, associated to the regulation of intra-cellular protein transport. In the present study, we device an integrated approach to understand these complex interactions. We analyze the gene perturbation expression profiles, to reconstruct a directed gene interaction network and decipher the regulatory interactions among genes, involved in protein transport signaling. In particular, we focus on gene expression signatures of genes involved in the secretory pathway of MCF7 breast cancer cell line. Further, network biology analysis approach delineates these gene-centric cross-talks, at the level of specific modules/sub-networks, corresponding to a different signaling pathways.

Session 3 :

Structure and Functions of Biomolecules

Structural and biochemical insights into p150 subunit of Chromatin Assembly Factor 1(CAF-1): a new tumor associated protein

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Chromatin assembly factor 1 (CAF-1) is a histone chaperone responsible for the positioning of histones into nucleosomes on newly synthesized chromatin. This heterotrimeric complex consists of three protein subunits: P150, P60, and P48. Despite the existence of many functional studies on this complex, a detailed description of the structural elements regulating the interaction of the three subunits within the complex is still missing. Recent data demonstrated that the overexpression of P150 is associated with the neoplastic progression of many human malignancies. Interestingly, expression levels of this protein significantly correlated with the biological aggressiveness of tumors, metastasizing behavior and worse prognosis. Taken together, these data indicate an important role of P150 in cancer progression; however, the molecular mechanism through which this occurs remains quite largely unknown and there is an emerging need to investigate the function of this novel tumor player. Based on these data, the aim of my Ph.D thesis is to gain insights into the biochemical and structural features of P150. To this aim, during the first year of activity, we approached a bioinformatic analysis to identify the functional domains contained within the full-length protein and we performed cloning and preliminary expression trials of these domains.

Imaging Mass Spectrometry of Lipids: from tissues to cells

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Over the last years, mass spectrometry (MS) has been improved into an innovative technology: Imaging Mass Spectrometry (IMS). IMS enables the correlation between **molecular information** and the **spatial localization** of the analytes, allowing multiplexed analysis of hundreds of compounds in the same analysis. In an IMS experiment, a laser beam 'scans' an area, collecting mass spectra for each coordinate and generating distribution images of ions by plotting the intensity of m/z ratio against the x-y coordinates.

The aim of this project is the analysis, localization and identification of lipids in different specimens: tissues and cells through the employment of the high-resolution AP-SMALDI10 instrument.

The first step of the work has been the development of suitable and reproducible protocols to detect and analyze lipids in both positive and negative ion modes, in mouse brain sections.

After this optimization phase, I analyzed lipids in individual cells (in particular fibroblasts) comparing images acquired with IMS with that of the stereomicroscope, to obtain a tentative IMS-optical imaging correlation method.

Another important aspect we evaluated is data interpretation. The large data sets obtained by IMS acquisition are, indeed, difficult to handle and require dedicated bioinformatics tools to be visualized in a meaningful form. For this reason, we have developed in collaboration with the IBP bioinformatics/ chemoinformatics Group, a dedicated software aimed at analyzing and visualizing IMS results for an easier interpretation.

Self-assembling proteins: GADD45 forms toxic amyloid-like aggregates under physiological conditions

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The uncontrolled self-assembling of proteins frequently leads to pathological states. Here we analysed the self-assembly propensity of the members of Growth Arrest and DNA damage-inducible 45 GADD45 family, denoted as GADD45α, GADD45β, and GADD45γ [1]. These proteins are involved in fundamental physio-pathological processes [2]. Since a full understanding of the their biological functions requires a detail characterization of their biochemical/biophysical properties we have undertaken structural studies on these systems. During these investigations we serendipitously found that GADD45β denaturates by forming aggregates that show a high content of β-structure. Further analyses that included binding experiments with the Thioflavin T dye clearly indicate that GADD45β forms amyloid-like assemblies. Since this protein unfolds at moderated temperatures (Tm ≈ 42 °C), these findings suggests that the protein may undergo this transition in physiological conditions.

The extension of these analyses to the other members of the family highlights analogies and differences. Indeed, GADD45α exhibits the same structural transition upon unfolding, although it occurs at higher temperatures (Tm ≈ 55 °C). On the other hand, GADD45γ, which shares a limited thermal stability with GADD45β, exhibits a reversible (partial) unfolding without forming any aggregate. Interestingly, cytotoxicity assays demonstrate that the amyloid-like assemblies of GADD45α and GADD45β are highly toxic against SH-SY5Y cells. The implications of these findings for the biological functions of these proteins will be discussed.

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Using zebrafish to assess the impact of bioactive metabolites from marine invasive species.

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Over the last decades, the spread of invasive species has become a critical multidisciplinary challenge. In environmental science, they represent a threat by competing with native species for space and resources, but also by producing bioactive compounds that may affect the local communities. In the Mediterranean basin, some green algae of the genus *Caulerpa* outstand as the most damaging invasive species encountered. The present research explores the effects of natural products extracted from two invasive *Caulerpa* species on model aquatic organism, with a special focus on caulerpin, an alkaloid found in *Caulerpa cylindracea*, and caulerpenyne, a sesquiterpene found in *Caulerpa disticophylla*. On one hand, caulerpin is known for its inhibitory effect on the P-glycoprotein mediating multixenobiotic resistance (MXR) mechanism in a marine sponge, while caulerpenyne has been recognized as a toxic compound. Our research focus on the study of caulerpin inhibitory properties through toxicity and biomolecular studies on the zebrafish (*Danio rerio*) as a model aquatic organism. Fluorescence measurement, and imaging of Rhodamine B in efflux assays are performed to clarify if caulerpin increase the vulnerability of zebrafish embryos to environmental toxic compounds, including *Caulerpa* toxins, such as caulerpenyne.

LC-MS^n-based polyphenol profiling of extracts from food by-products with nutraceutical and cosmeceutical value

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The project will perform activities focused on the recovery of high added-value polyphenol components from food and its by-products for the formulation of new dietary and healthy products as nutraceuticals and cosmeceuticals. These latter have become a prime focus in the health and well-being market nowadays, bolstered by the growing population of health-conscious consumers, and polyphenols, pure or in mixture form, are attractive their ingredients. In fact, these compounds, virtually present in each plant part and its products, are known to prevent or slow-down oxidative stress related diseases and/or disorders thanks to their attitude to neutralize, deactivate or suppress free radical species or, directly, to act as inhibitors of lipoperoxidation. In particular, in the first year of the PhD course the nutraceutical and cosmeceutical potential of hempseed and grape products and by-products will be investigated. The optimization of extractive procedures will be addressed to the full recovery of the targeted compounds. The metabolic profiling of the extracts obtained will be performed using LC-MS analyses. The extracts' antioxidant capability will be evaluated by several methods under different oxidative conditions, whereas cytotoxicity/cytoprotection assays, which use different parameters associated with cell death and proliferation, will be performed.

Untargeted Metabolomics evaluation of nutraceuticals using NMR as main analytical platform

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Nutraceuticals are food or part of food that provides medical or health benefits including the prevention and/or treatment of a disease, is well known in fact these products combines nutrient and pharmaceutical capabilities. The principal aim of nutraceuticals is to focus on prevention, according to the idea of Hippocrates saying "let the food be your medicine". Principal benefits of nutraceuticals are: avoiding side effects, increasing the health beneficial effects, having naturally dietary supplement, increasing availability and affordability. Metabolomics is the study of the metabolite composition of cell type, tissue, or biological fluid. Metabolites are the intermediates or end products of multiple enzymatic reactions so they represent the key to understand the biochemical activities of an organism. In this project, we try to focus on the study of metabolomics profiles of potentially nutraceutical products thanks to nuclear magnetic resonance (NMR) spectroscopy. NMR is fast and highly reproducible spectroscopic technique based on energy emission of atom nuclei under the influence of an external magnetic field. Beyond the capability to allow the quantification of the concentration of metabolites, NMR spectral data also provides information on the molecules chemical structure. Due to considerable diversity in the matrices of potential nutraceutical products, different extraction methods and sample preparation techniques will be used and benchmarked using NMR as main analytical platform. Furthermore, all experimental data will be statistically processed for interpretation.

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Mechanisms of BARS-mediated mitotic Golgi fragmentation

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One of the control mechanisms that regulate the correct cell cycle progression is the so-called "Golgi mitotic checkpoint". During mitosis the Golgi complex undergoes extensive fragmentation through a multistage process that allows its correct partitioning into the daughter cells. This process is required not only for correct cell inheritance, but also for mitotic entrance itself, since its block results in the arrest of the cell cycle in G2 phase. We have identified CtBP1-S/BARS as key controller of the Golgi ribbon unlinking during mitosis. Moreover, CtBP1-S/BARS controls also the membrane fission processes required during several intracellular trafficking pathways such as: formation of basolateral post-Golgi carriers, fission of COPI-coated vesicles, macropinocytosis and fluid-phase endocytosis.

The CtBP1-S/BARS-complex components involved in membrane fission have been identified and characterized. This complex comprises CtBP1-S/BARS bridged to PI4KIIIβ by a 14-3-3γ dimer and includes also ARF, PLD1/2 and the two stabilizing kinases PKD and PAK. Once incorporate into this complex, CtBP1-S/BARS binds to and activates two Golgi localized lysophosphatidic acid (LPA) acyltransferase enzimes, namely LPAAT3 and LPAAT4. In order to define the molecular mechanisms underlying the CtBP1-S/BARS-mediated Golgi fragmentation during mitosis, we are studying the role of these components in cell-cycle synchronized HeLa cells. The specific depletion of CtBP1-S/BARS, or LPAAT3, or LPAAT4, or PAK1/2, or PLD1/PLD2 complex-components strongly inhibits the mitotic Golgi fragmentation (although to different extents) revealing the relevance of this CtBP1-S/BARS protein-complex in the mitotic Golgi partitioning.

Biological activities of the ribotoxin Ageritin from *Agrocybe aegerita* (V. Brig.) Singer

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During the first PhD year, a basic protein (17 kDa), hereafter Ageritin, with the ability to inhibit protein synthesis *in vitro* (IC_{50} value of 133 pM) was purified to homogeneity from the edible mushroom *Agrocybe aegerita* (Basidiomycota phylum). Therefore, during the second year, a specific enzymatic characterization was carried out for Ageritin (1). Several assays were performed (RNAse, DNAse and protease activity), but Ageritin was only positive to Endo's assay showing that it induces a specific cleavage of a single phosphodiester bond, with and without aniline treatment, located at the universally conserved sarcin/ricin loop of the large rRNA (SRL region). This behavior is well documented for ribotoxins as α-sarcin (2). Thus, the finding allows us to conclude that Ageritin was a novel member of this RNase class. These observations, together with literature consultation, made it clear that Ageritin was the first ribotoxin isolated from Basidiomycota phylum, suggesting that these enzymes could be more widely distributed among fungi than previously believed (1). Finally, the cytotoxicity of Ageritin was assayed on several CNS model cell lines (SK-N-BE(2)-C, U-251 and C6) or against the green mold *Penicilium digitatum* (antifungal activity).

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Metal ion replacement by Pb(II), Ni(II) and Hg(II) in the prokaryotic zinc-finger domain

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Zinc ion binding to the proteic domain is a principal event in the achievement of the correct fold in the classical zinc fingers domain since the motif is mainly unfolded in the absence of the metal cofactor. However, in the case of prokaryotic zinc finger the bigger bbbaa domain shows a hydrophobic core larger than the one found in eukaryotic zinc fingers and that plays a more relevant role in the folding mechanism. For these reasons, as great attention has been devoted to unveil the effect of metal ion replacement in zinc fingers and in zinc-containing proteins in general, the prokaryotic zinc finger domain appears to be a good model to study the interaction of exogenous metal ions with metallo-proteins.
We here explore the structural and functional consequences of the native Zn(II) substitution by Ni(II), Pb(II) and Hg(II) in Ros87, the DNA binding domain of the prokaryotic zinc finger protein Ros. Our findings will complement and extend previous results obtained for different eukaryotic zinc fingers, contributing to the evaluation of whether metal substitution in zinc fingers may be a relevant mechanism in the toxic and/or carcinogenic effects of metal ions.

Structural characterization of proteins belonging to the prokaryotic zinc finger family: Ml1 from *M. loti.*

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Tutor: Roberto Fattorusso
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The possibility of choices of protein ligands and coordination geometries leads to diverse Zn(II) binding sites in zinc-proteins, allowing a range of important biological roles. The prokaryotic Cys_2His_2 zinc finger domain (found in the Ros protein from *A. tumefaciens*)[1] tetrahedrally coordinates zinc thorugh two cysteine and two histidine residues and unfolds in the absence of the metal ion[2]. It is the first structurally characterized member of a new family of bacterial proteins[3] that presents several amino acid changes in the positions occupied in Ros by the zinc coordinating residues[4]. In particular, the second position is very often occupied by an aspartic acid. Since the coordination of structural zinc by an aspartate in a zinc finger is very unusual, to also elucidate whether and how Ros homologues bind the zinc ion when the coordinating cysteine is replaced by an aspartate, we here report the characterization of Ml1 from *M. loti*, a Ros87 homologue that bears a $CysAspHis_2$ coordination sphere.
UV-Vis, CD and NMR techniques are used to characterize the structure, metal binding affinity, dynamic behavior and folding pathway of this protein. The data obtained are compared to those collected on the protein Ros in the last few years demonstrating how the presence of an aspartic acid in the metal ion coordination sphere, while preserving the overall architecture of the domain, leads to the switch from the folding pathway observed in Ros87 to a different folding pathway. Ros87 and Ml1 are also compared in terms of aggregation properties in order to evaluate the influence of the different folding pathways on the fibril formation propensity of the two examined proteins.

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NMR characterization and antiproliferative activity of secondary metabolites from Fabaceae species

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Compounds isolated from several Fabaceae species have antitumor properties against different tumor types, including colorectal cancer which is estimated to be the third most common type of cancer in the United States. Current chemotherapeutic agents used for the treatment of colorectal cancer are not entirely efficient to date due to the side effects of the drugs and the development of resistance to them. Thus, there is a renewed interest for the natural products, as an alternative therapy to cure colorectal cancer patients. In this work, the NMR-based metabolomic screening of 14 Fabaceae extracts has been performed as well as their anti-proliferative assessment. As a result three plant extracts (*Astragalus boeticus*, *Ononis diffusa* and *Trigonella corniculata)* have been selected for their cytotoxic activity against a panel of different colon cancer cell lines, including those resistant to the conventional drugs. Consequently, a targeted phytochemical study of these extracts quickly allowed to isolate the compounds responsible for the anti-proliferative activity. Thanks to an extensive NMR analysis (1D and 2D experiments) in combination with TANDEM MS techniques, the chemical structure of these has been elucidated. Finally, attempting to understand the metabolic changes caused by the isolated compounds, experiments to investigate the metabolome and proteome of the studied colon cancer cell lines, before and after the treatment, are in progress.

Novel highly constrained peptide ligands for modulating the activity of Cripto-1 and Activin-like receptors.

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Cripto-1, the founding member of the extra-cellular EGF-CFC family of growth factors, is an oncogenic protein which performs key functions in embryonic development, stem-cell differentiation and tumourigenesis [1]. The protein is composed of two cysteine-rich domains of approximately 40 aminoacid residues, the CFC and the EGF-like, playing distinct functional and structural roles. The EGF-like domain which bind Nodal, whereas the CFC domain which binds ALK4 [2]. Assembly of this complex, ALK4/Cripto/Nodal/ActRIIB does support uncontrolled cell growth and proliferation through signaling pathways involving the Smad2/3 and Src/MAPK/PI3K intracellular mediators [3]. In the attempt to obtain new more specific and potent Cripto-blocking peptides, useful to inhibit of cell growth and for the treatment of various cancers, we have used a structure-based approach starting from the CFC domain structure and introducing residues crucial for Cripto-ALK4 recognition [1]. Linear peptides were prepared by SPPS, cyclized around a tri-bromo-methylbenzene (TBMB) scaffold [4] and tested for binding to ALK4 by SPR assays. By this screening, we have identified a set of bicyclic peptides that have been tested on different lines of breast cancer and melanoma cells known to express Cripto-1 and to have Cripto-dependent proliferation features [5, 6]. The peptides show cell-killing activity on selected cell lines although potency is apparently reduced compared to the precursor synthetic CFC domain. Activity does correlate with in vitro binding data and with structural features obtained by CD and NMR.

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Brain D-amino acids metabolism in neurological disorders

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The endogenous D-amino acids D-serine and D-aspartate are, respectively, co-agonist and agonist of N-methyl D-aspartate receptors (NMDARs), and play important roles in physiological and pathological processes mediated by this subclass of glutamate receptors. Based on the hypothesis of NMDAR hypofunction in the pathophysiology of schizophrenia, we assessed the concentration of both D-serine and D-aspartate in *post-mortem* dorsolateral prefrontal cortex (DLPFC) and hippocampus samples, from healthy subjects and patients with schizophrenia. Interestingly, we found a selective reduction of D-aspartate content in DLPFC of psychiatric patients, coupled to an increased cortical activity of D-aspartate oxidase (DDO), the enzyme responsible for the D-aspartate catabolism. Abnormalities in NMDAR-mediated transmission have been also associated to Parkinson's disease (PD). Therefore, we investigated D-serine and D-aspartate metabolism in the brain of non-human primate *Macaca Mulatta* PD model, lesioned with the neurotoxin MPTP, or supplemented with chronic L-DOPA. Results showed that striatal levels of D-aspartate and D-serine increased after dopamine depletion, and were normalized following L-DOPA treatment. Conversely, we did not find significant changes in the transcription of genes responsible for D-serine and D-aspartate metabolism. These results reveal that altered brain D-amino acids metabolism may represent a factor contributing to dysfunctional NMDAR-mediated transmission in schizophrenia and PD pathologies.

Advanced mass spectrometry-based proteomics strategies for probing protein complexes and interaction networks.

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The characterization of protein complexes and the elucidation of protein-protein interactions by mass spectrometry (MS) is an increasingly important part of post-genomics strategies to understand protein functions. Herein, we describe two strategies based on chemical crosslinking and Biotin Identification (BioID) proximity labeling coupled with MS for mapping protein-protein interactions *in vitro* and *in vivo*, respectively, on two experimental systems.

Photo-chemical crosslinking labeling methodologies[1] coupled to nano LC-High Resolution MS/ MS have been used to probe the interaction site of the Gadd45β/MKK7 complex *in vitro*, whose formation plays a key role in the onset of Multiple Myeloma in individuals expressing high levels of Gadd45β[2]. We found that interactions are mediated by two complementary surfaces, one involving residues 396-405 of MKK7 and 116-131 of Gadd45β, the other involving residues 102-112 of MKK7 and 92-97 of Gadd45β. Furthermore, interaction sites of kinase with the DTP3 inhibitor[2] have been also identified on MKK7 regions 113-136 and 259-274, which are spatially adjacent and form a shallow cavity close to the catalytic pocket.

Furthermore, by using a proximity-dependent BioID[3] approach coupled to nanoLC-high resolution tandem MS, the proximal interactome of the Zinc-finger Telomere-Associated Protein (TZAP), involved in telomere length regulation[4], has been mapped in Hela cells. Over 63 potential interactors have been identified. Selected interactors have been validated, including Ki-67 and BAZ1B, thus demonstrating that TZAP interacts with nuclear proteins of the chromatin remodeling complex family.

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Biochemical studies of PON2 and its variants, a protein strongly implicated in in development of metabolic diseases

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It is well established that oxidative stress from mitochondria plays an important role in apoptosis and also leads to premature aging and cancer. There is growing scientific consensus that proteins with antioxidative functions, such as paraoxonases, can lower the incidence of these diseases.

The human paraoxonase 2 (PON2) has been described as highly specific lactonase hydrolysing the quorum sensing molecule N-(3-oxododecanoyl)-Lhomoserine lactone (3OC12-HSL) and having secondary esterase activity. PON2 is an intracellular protein, expressed in a wide range of cell types, including pancreatic beta cells. Importantly, polymorphisms in the PON2 protein (S311C and A148G) have been associated by several studies with diabetes and its complications. An engineered version of PON2 wt and the polymorphic variants have been over-expressed in *E. coli,* refolded from inclusion bodies and purified, yielding an enzyme with the same characteristics as the full length enzyme. A biochemical characterization has been performed, confirming the primary activity on 3OC12-HSL for the wt and a decrease for the mutants. This could be relevant to diabetes type 2 and cardiac failures that have been related to these SNPs.

The enzymes produced in *E.coli* are fully active but not very stable probably because using a prokaryotic expression system the post-translational modifications of the native form are not present, such as glycosylation that affects enzyme stability. So PON2 was produced in insect cells obtaining a more stable enzyme that is undertaken for crystallizazion trials because the structural information will permit to compare the effects of wild type and mutant variants chosen according to structural insights and biochemical informations.

Session 4 :

Human Genetics

Alzheimer's disease (AD) and Frontotemporal dementia (FTD): investigating the overlap of genetic mutations focusing on *CD33* and *TREM2*.

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Frontotemporal dementia (FTD) and Alzheimer's disease (AD) are two complex neurodegenerative disorders with associated several factors, including the immune genes *CD33* and *TREM2*, suggesting that a common molecular pathway could exist and is yet unknown.
Two *CD33* SNPs and one in *TREM2* were described to be predisposing factors to Late Onset Alzheimer disease (LOAD). In particular, *CD33* SNPs rs3865444 and rs12459419 directly modulate *CD33* exon 2 splicing efficiency. Moreover, the variant rs75932628-T in *TREM2* exon 2 was strongly associated with the capacity of TREM2 to activate microglial cells.
In order to assess the presence of these polymorphisms in our cohort, we performed High Resolution Melting analysis (HRM) on genomic DNA of 216 Caucasians diagnosed with LOAD and 50 healthy controls.
Our patients exhibited the coinheritance of SNPs rs3865444 and rs12459419. In addition, we identified a third SNP in *CD33* exon 2, rs2455069, which belongs to a LD SNP block associated with an increased rate of cognitive decline which we found in an unusual family with phenotype AD/FTD. We found that all patients analyzed for SNP rs75932628 in *TREM2* gene are homozygous for the wild-type allele. Further investigations are in progress to understand the mechanism of action of these two genes.

Dissecting the molecular mechanism underlying Paget's Disease of Bone complicated by Osteosarcoma

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Paget's disease of bone (PDB) is a late onset bone turnover disorder, caused in most of the cases by mutations in *SQSTM1* and, rarer, in *ZNF687*. However, several PDB patients negative for mutations have been reported, suggesting the involvement of other genes. The malignant transformation of PDB bones is a rare but feared complication, with osteosarcoma having a 5-year survival rate almost nil.
Applying whole-exome sequencing on a family with 10 members with early onset form of PDB, 3 of whom also developed osteosarcoma, we identified 4 candidate genes (*CAMTA2*, *GGT6*, *MYH3* and *PFN1*), all playing some role in bone biology. Among them, we started functional experiments on *PFN1*, given the presence of a heterozygous frameshift mutation. qRT-PCR demonstrated that the mutant *PFN1* mRNA doesn't undergo nonsense-mediated decay. Conversely, western blotting on patients' lymphoblasts detected a reduced expression of mutant profilin-1 compared to control. In agreement, MG132 treatment on cells expressing the truncated protein revealed its partial degradation by the proteasome and the formation of cytoplasmic aggresomes. These results suggest that abnormalities in protein clearance may contribute to the onset of the disease.
Further studies are on-going to disclose the role of the other candidate genes in the pathogenesis of pagetic osteosarcoma.

CRIPR-Cas9 genome editing to unravel the role of PGRN in neuronal cells.

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Progranulin (*GRN*) gene is one of the major genetic determinants of Frontotemporal Dementia (FTD) and mutations are present in 23% of familial cases. The neurobiology of the PGRN protein remains still unclear although the proposed disease mechanism is neuronal deficiency.

We performed a genetic screening on 256 FTD patients and 300 healthy, age-, sex- and geographic region-matched controls and identified a rare *GRN* gene exon six deletion g10325_10331delCTGCTGT (relative to nt 1 in NG_007886.1), alias Cys157LysfsX97 in three autosomal dominant Southern Italian pedigrees. Gene expression analysis on mRNA from WBC and proteins from plasma of FTD patients carrying the mutation showed deficiency of the protein.

To gain a system level view of the molecular consequences of PGRN depletion we used CRISPR-Cas9 to generate a cellular model of *PGRN* deficiency.

To begin testing the hypothesis that patients with PGRN deficiency have an impaired autophagic-lysosomal pathway, we performed mRNA expression analysis of autophagy related genes. Preliminary results demonstrated decreased expression of autophagy-related genes *Beclin2* and *LC3* in *PGRN* knock-out compared to wild-type cells. Our observations support the role of the *GRN* gene in the etiology of FTD and the hypothesis that PGRN insufficiency can predispose to neuron degeneration.

Identification and characterization of molecular defects in congenital growth-related imprinting disorders.

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Less than 1% of human genes are imprinted by epigenetic mechanisms and as a result, their expression is monoallelic and parental origin dependent. Two different Imprinting Control Regions (ICR1 and ICR2) regulate the expression at one of the major cluster of imprinted genes, located on 11p15.5 chromosome. The cluster harbours most of the molecular defects associated with the overgrowth-related Beckwith-Wiedemann Syndrome, characterized by heterogeneous molecular defects and clinical features. In about 15% of the patients the molecular defect is still unknown. Also the (epi)genotype-phenotype correlation for a correct prognosis is restricted to a few features.
To identify novel molecular defects we have performed targeted sequencing of the entire 11p15.5 imprinted cluster in 89 BWS patients. Genomic DNA has been processed by targeted capturing, library preparation and sequencing. A pipeline has been designed to obtain a list of unknown variants. Bioinformatics analysis to identify putative clinical variants is ongoing.
We found two families carrying mutations in *KCNQ1* associatated to hypomethylation of ICR2, BWS and the heart rhythm condition Long QT syndrome, suggesting molecular defects common to both pathologies.

Session 5 :

Cancer biology, Immunology, Microbiology, Drug design

Retro-inverso Urokinase Receptor Antagonists for the Treatment of tumor metastasis.

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The urokinase receptor (uPAR) promotes cell migration by interacting with the Formyl Peptide Receptor type 1 (FPR1). Since both uPAR and FPR1 are involved in tumor progression, the uPAR-FPR1 interaction is an attractive therapeutic target. We previously described peptide antagonists of the uPAR-FPR1 interaction that inhibited cell migration. To develop enzyme-resistant analogues, we applied the Retro-Inverso (RI) approach, whereby the topology of the side chains is maintained by inverting the sequence of the peptide and the chirality of all residues. A number of RI peptides were generated and preliminary analyzed for their ability to affect cell migration. Among these, the peptide Ac-(D)-Tyr-(D)-Arg-Aib-(D)-Arg-NH2 (RI-3) was selected as the best competitor of N-formyl-Met-Leu-Phe for binding to FPR1. RI-3 adopts the turn structure typical of the uPAR-FPR1 antagonists. RI-3 is a nanomolar and inhibits migration, invasion, and trans-endothelial migration of sarcoma cells (IC50 in the pM range). In vivo, daily administration of RI-3 reduced vascular infiltration by human sarcoma cells in nude mice. Thus, RI-3 represents a promising lead for developing novel anti-metastatic drugs. Future purpose is to investigate the effects of RI-3 on tumor cells in 3D organotypic co-cultures with stromal cells.

Role of Neuroendocrine Transdifferentiation in Prostate Cancer Development and Drug Resistance

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Prostate adenocarcinoma (PCa) is the second leading cause of male cancer death. Androgens regulate PCa tumorigenesis and, although hormone therapy is very effective, it can switch to hormone independent and drug resistant status. Neuroendocrine differentiation (NED) of PCa cells impact on cancer progression and therapeutic responses, but its mechanism is still controversial.
The project aims to explore the key factors that drive NED and androgen independence focusing on the potential involvement of the endocannabinoid system (ES) and transient receptor potential (TRP) channels in order to develop new therapeutic strategies for hormone-refractory PCa.
We used *in vitro* model of murine PCa (TRAMP-C2 cells) and in vivo multistage model of prostate cancer (TRAMP mice), which uniformly and spontaneously develops autochthonous prostate tumors. Additionally, we induced NED in TRAMP-C2 cells (TRAMP-derived prostate tumor cells) under prolonged androgen deprivation (7 days) and we set up *in vivo* model of hormone refractory prostate with TRAMP mice. The effect of phytocannabinoids on TRAMP-C2 cell viability and different stages of tumor in TRAMP mice is currently under investigation as well as mechanism(s) of action.
Further work will investigate NED, ES and TRP channels in fluids and tissues derived from PCa in vivo model to identify novel biomarkers.

Structure-Based Virtual Screening for the search of novel SMO antagonists: a ligand repurposing approach

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Dysregulation of the Hedgehog Signaling Pathway is involved in the development of a wealth of solid tumors, as it has been well established. In particular, the aberrant activation of this pathway is linked to metastasis growth and acquisition of resistance to traditional chemotherapeutic agents. The GPCR-like receptor Smoothened (SMO) is part of this pathway and represents an attractive target to antagonize in cancer treatment. Thus, we devised an in silico protocol which is fine-tuned to identify new potential ligands for this receptor and coupled this method with the intrinsic advantages of a drug repurposing approach. Such a protocol employs the docking software AutoDock Vina, which allows for fast and comprehensive virtual screening campaigns. To probe the predictive power of our method, we screened a database of inhibitors active against the tyrosine kinase MET, whose overexpression is also heavily implied in cancer progression. The most promising hits resulting from this campaign proved to be active in the nanomolar range in biological assays against SMO, representing the first dually-active ligands against these two structurally different targets.

Pharmacological chaperones to cure genetic diseases.

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Fabry Disease (FD) is a rare genetic disease caused by mutations in the *GLA* gene. There exist more than 400 missense mutations of *GLA*, causing the disease, that have a different effect on the stability and the activity of the enzyme with the consequent difficulty to find a suitable therapy. We are focused on a possible therapy that uses little molecules called pharmacological chaperones (PC). PCs are small molecules that are able to bind the mutated enzyme, stabilizing it and increasing its concentration and then the enzymatic activity within the cell. The PC, almost approved, that improves patient's conditions in FD, is called DGJ (1-deoxigalactonojirimycin). We gathered data from several independent research groups of enzymatic activity of *GLA* mutants in presence or absence of DGJ and made them comparable. We correlated data obtained from different transfected cells with those obtained in cells derived from patients, and finally evaluated the possible response of every mutant to DGJ by *in silico* tools to facilitate the choice of eligible patients. The aim of this meta-analysis study is not only to bring stronger statistical conclusions then a single research, but also to demonstrate the good reliability of *in vitro* methods that well represent the residual activity in patients, and finally make a step forward for a personalized therapy for patients according the owned mutation.

Inhibition of bone marrow-derived mesenchymal stem cells homing towards triple-negative breast cancer microenvironment using an anti-PDGFRβ aptamer

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Bone marrow-derived mesenchymal stem cells (BM-MSCs) are shown to participate in tumor progression by establishing a favorable tumor microenvironment (TME) that promote metastasis through a cytokine networks. However, the mechanism of homing and recruitment of BM-MSCs into tumors and their potential role in malignant tissue progression is poorly understood and controversial. Here we show that BM-MSCs increase aggressiveness of triple-negative breast cancer (TNBC) cell lines evaluated as capability to migrate, invade and acquire stemness markers. Importantly, we demonstrate that the treatment of BM-MSCs with a nuclease-resistant RNA aptamer against platelet-derived growth factor receptor β (PDGFRβ) causes the inhibition of receptor-dependent signaling pathways thus drastically hampering BM-MSC recruitment towards TNBC cell lines and BM-MSCs trans-differentiation into carcinoma-associated fibroblast (CAF)-like cells. Moreover, *in vivo* molecular imaging analysis demonstrated the aptamer ability to prevent BM-MSCs homing to TNBC xenografts. Collectively, our results indicate the anti-PDGFRβ aptamer as a novel therapeutic tool to interfere with BM-MSCs attraction to TNBC providing the rationale to further explore the aptamer in more complex pre-clinical settings.

Role of a mycobacterial TetR regulator in stress response.

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Mycobacterium tuberculosis is a pathogen able to survive under acid-nitrosative stress inside the macrophages. Previous studies indicated that the expression profiles of *M. tuberculosis* and *Mycobacterium smegmatis,* exposed to acid-nitrosative stress, show up-regulation of *M. tuberculosis Rv1685c* and of its orthologue in *M. smegmatis, MSMEG_3765*. Both genes are annotated as TetR transcriptional regulators. Microarray analysis, conducted on *M. smegmatis* wild type and *ΔMSMEG_3765* strains, show that the TetR-MSMEG_3765 protein regulates the expression of genes involved in a wide range of cellular activities, including biosynthesis of antibiotics, multidrug resistance and efflux pumps. Among these, the transcriptional profile showed a TetR-MSMEG_3765-mediated down-regulation of the *MSMEG_3762* and *MSMEG_3763* genes, annotated as ABC transporter ATP-binding protein and ABC transporter (efflux pump components), respectively, and a TetR-MSMEG_3765-mediated up-regulation of *MSMEG_4765*, annotated as a MerR transcriptional regulator; many regulators of this family respond to environmental stimuli, such as oxidative stress, heavy metals or antibiotics. I'll focus my research work on the role of these genes in response to stress conditions often encountered by *M. tuberculosis* within macrophages; the complexity of the stress response is functional to prevention of phagosome maturation, antigen processing and apoptosis, and to formation of an environment permissive for bacterial metabolism and growth.

Optimization of adoptive T cell therapy by promoting the correct pairing of T cell receptor chains

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The adoptive therapy with T cell receptor (TCR)-engineered T cells has shown promising results in the treatment of cancer patients. It is based on the redirection of T cells specificity towards tumor-associated antigen, and its weakness is the mispairing between the endogenous and introduced alpha and beta TCR chains that limits the expression of the therapeutic TCR impairing the efficacy of the therapy and resulting in off-target reactivity or autoimmunity.
Our goal is to promote the correct pairing of transduced TCR chains by introducing aminoacidic mutations in the transmembrane regions of TCR chains. We already demonstrated, in a mouse model, that the proposed strategy avoids TCR mispairing and increases T cells functional activity. During this second year of my PhD we translated this strategy to human TCRs by adding additional mutations in the membrane proximal region of both a and b chains. These mutations were introduced in the 1G4 Ny-ESO-1 TCR, which has high inter-chain affinity and it is expressed at maximal levels on transduced T cells. We observed an increase in the signal transduction activity of the mutated TCR. Now, we are selecting TCRs with weaker pairing properties to analyze the benefit given by the introduction of our mutations.

MET-AS: a novel long non-coding RNA that regulates MET in papillary thyroid carcinoma

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Papillary thyroid carcinoma (PTC), the most common type of thyroid cancer (~80%), is mainly caused by mutually exclusive *BRAF* and *RAS* gain of function somatic mutations.
BRAF- and *RAS*-mutated patients, and those with similar transcriptional profiles (*BRAF*- and *RAS*-like) have distinct gene expression signatures, considering both protein-coding and long non-coding RNAs (lncRNAs). The latter are indicated as new candidates in oncogenic and metastatic processes as well as in drug resistance. Among differentially expressed lncRNAs in our PTC RNA-Seq datasets, we focused on a new lncRNA, *MET-AS,* transcribed on the opposite strand of *MET* oncogene. The aim of my Project is to address *MET-AS* oncogenic capability in papillary and anaplastic thyroid tumors.
During my project, I found that the newly identified lncRNA and its neighbour *MET* are significantly over-expressed in the most aggressive PTC subgroup, i.e. *BRAF*-like, compared to *RAS*-like and healthy controls. RNA fractioning and RNA FISH in PTC cell lines defined it is a cytosolic lncRNA. Functional assays revealed that its siRNA-mediated knockdown impairs MET mRNA and protein levels. Additionally, its silencing significantly reduces proliferation, viability and colony forming capability, as well as migration and invasiveness of thyroid cancer cell lines, in line with MET involvement in the invasive growth program. Moreover, in order to investigate the reason why *MET-AS* is highly-expressed in *BRAF*-like tumors, based on computational evidences, knockdown of BRAF and AP1 complex members are carrying out. Further studies are still in progress to mechanistically define how *MET-AS* how it exerts its oncogenic role.

Modulation of tumor microenvironment: new decapeptides inhibiting human fibroblasts pro-tumoral activity

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Progression of solid tumors not only depends on the complex events initiated by oncogenic lesions, but also on cancer cell ability to recruit a variety of stromal cells and render them supportive of tumor growth and dissemination. Among stromal cells, Cancer-Associated Fibroblasts (CAF) are regarded as major facilitators of tumor proliferation, survival, invasion and metastasis. New anti-cancer strategies are arising, aiming to concurrently co-target neoplastic cells and CAF.
This work is focused on the effects of two novel deca-peptides, targeting αvβ5 integrin and denoted DV1 and DV2 on the pro-tumoral activity of CAF from breast cancer patients. Following cell exposure to nanomolar concentrations of either peptides, proliferation of human dermal fibroblasts (TIF) was not affected. In contrast, peptide-treated-TIF or primary CAF from breast carcinoma patients (intra- or peri-tumoral) exhibit a decreased α-SMA and increased Caveolin-1 protein levels, both well known CAF markers. These effects, counteracting CAF phenotype are accompanied by a reduced pro-invasive ability in 3D-organotypic co-cultures, when tested in combination with either HT1080-GFP fibrosarcoma or MDA-MB-231 mammary tumor cells. In these conditions, peptide-exposed CAF exhibit a reduced capacity to remodel collagen matrix, and to secrete chemoattractants to mobilise tumor cells. Finally, in a mouse model of lung colonization, using HT1080 cells injected in the tail vein, DV2 peptide reduces the number and the size of metastases, showing a remarkable anti-metastatic activity *in vivo*. In conclusion, these data indicate that the novel peptides modulate CAF activation, counteracting the pro-tumoral activity of stromal fibroblasts.

Expression and Functional Characterization of Ultraconserved Non-Coding Region 8+ in Bladder Cancer

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Transcribed ultraconserved regions (T-UCRs) represent a group of highly conserved sequences among orthologous regions of human, rat and mouse genomes. They represent a new class of long non-coding RNAs (lncRNAs) whose function is still unknown. While microRNAs and other types of lncRNAs have been shown to contribute to the biological function of bladder cancer (BlCa) and are increasingly being used to improve the clinical care of patients, this is not yet the case for TUCRs. By using genome-wide profiling, we identified 293 T-UCRs de-regulated in BlCa patients as compared to normal tissues. T-UCR8+ is the most up-regulated and inversely related to grade, paving the way for clinical applications. In vitro experiments evaluating the effects of T-UCR8+ silencing showed significantly decreased capacities for cancer cell invasion, migration, and proliferation. For this reason, we proposed and validated an interaction model in which T-UCR8+ shuttles from the nucleus to the cytoplasm of BlCa cells, interacts with microRNA (miR)-596, and cooperates in the promotion and development of BlCa. By using computational analyses, we investigated the miR-binding domain accessibility, as determined by base-pairing interactions within the T-UCR8+ predicted secondary structure, RNA binding affinity, and RNA species abundance in bladder tissues and showed that T-UCR8+ is a natural decoy for miR-596. Thus T-UCR8+ up regulation results in increased expression of MMP9, increasing the potential invasion of BlCa cells. To better clarify the cytoplasmic function of T-UCR8+ during tumorigenesis and understand its role, we dissect T-UCR8+ protein network interaction using the RAP–MS (RNA-antisense purification-mass spectroscopy) method. As preliminary data, we purified T-UCR8+ sequence and we are now performing the mass spectroscopy to verify which proteins bind to this lncRNA. To confirm its localization, we also performed ISH (*In situ* Hybridization) experiments in BlCa tissues and different embryo stages.

Glioblastoma: molecular compounds targeting tumor cells

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Glioblastoma Multiforme (GBM) is an aggressive high-grade glioma (grade IV). Aggressive invasiveness is major pathobiological characteristic of GBM. GBM depends on vascular networks to supply blood, oxygen, and nutrients. Tumor blood vessels can either be formed from pre-existing blood vessels (neo-angiogenesis) or from tumor cells (vasculogenic mimicry); vascular mimicry provides a mechanism whereby GBM could escape anti-angiogenic therapies.
In my PhD project I'm evaluating whether HDAC inhibitors and Ruta extract are able to affect proliferation, motility and vasculogenic mimicry in glioma cells.

I tested SAHA (Varinostat) and trichostatin A (TSA) as inhibitors of class I and II HDACs, MS275 (Entinostat) as selective inhibitor of class I HDACs and MC1568 as selective HDAC class II inhibitor.
At no toxic concentration of 50nM, TSA, MS275 and MC1568 HDACis significantly decrease U87MG directional cell migration in Boyden chamber assays and we found that HDACi MS275 is able to impair U87MG matrigel invasion.
In Tube Assay, all HDACis studied inhibit vasculogenic mimicry in U87MG, whereas only SAHA and MC1568 inhibit significantly vasculogenic mimicry in C6 cell lines.

Ruta extract is able to affect cell proliferation, cell motility and vasculogenic mimicry.
Results suggest that HDACis and Ruta extract may be promising candidates for GMB therapies

Prognostic Value of Left Ventricular Shape Index Assessed by Gated SPECT Myocardial Perfusion Imaging

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Aim. Left ventricular (LV) remodeling is a subset of compensatory changes of myocardium and it is associated with adverse cardiovascular events. Quantitative indexes of 3-dimensional LV geometry, such as LV shape index (SI), can be obtained automatically by gated SPECT program. This study sought to assess the prognostic value of LVSI in patients without coronary artery disease (CAD) and normal LV function.
Materials and Methods. We prospectively evaluated 646 patients with suspected CAD with normal myocardial perfusion and LVEF at gated-SPECT. An automated software program calculated LV volumes, LVEF, and LVSI (end-systolic normal value: ≤0.54).
Results. Median follow-up was 37 months. In 646 subjects, 25 cardiac events occurred with cumulative event rate of 3.8% and an incidence rate of 1.25 per 100 persons/year (95% confidence interval 0.85-1.85). Patients with events were older, had higher prevalence of diabetes and higher LVSI values as compared to patients without events (all $P<0.001$). At multivariable Cox-regression analysis, age ($P<0.01$) and end-systolic LVSI ($P<0.001$) resulted independent predictors of cardiac events. At incremental analysis, end-systolic LVSI added prognostic information in a model including clinical variables, increasing the global chi-square ($P <0.001$). At the event-free survival analysis, patients with abnormal end-systolic LVSI had the worst outcome ($P<0.001$).

“Exploitation of new strains for drug discovery from deep sea sediments”

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Introduction Currently, multi drug resistant infections are a primary concern for the WHO and society as a whole. Microorganisms, such as human pathogenic bacteria have developed resistance to antibiotics primarily through the misuse and overuse of these drugs. Extreme environments such as the Antarctic, harbour a diverse range of microorganisms that have over the course of evolution developed molecular adaptations to these cold environments. The unique metabolism of these microorganisms includes secondary metabolites that have been reported to possess several bioactivities, including anti-microbial and anti-biofilm activities. In this PhD study, microbial diversity and metabolic potential of Antarctic deep sea sediments, are assessed for pharmaceutical and biotechnological applications. **Methods** Fast-growing bacteria were isolated using culture-dependent techniques such as varying incubation time, temperature, and culture media, which was followed by preliminary bioactivity screening by cross-streaking method. Isolates of interest were then subjected to small-scale fermentations, and crude extractions using organics solvents. Extracts were assessed for anti-microbial activity using minimum inhibitory concentration (MIC) assay. **Results** Fifty bacteria have been isolated using culture-dependent techniques. Nine isolates showing preliminary bioactivity were subjected to fermentation, and ethyl acetate extraction. Crude extracts assessed showed MIC values of greater than 200 µg/mL. **Conclusion** The remaining isolates will be subjected to the bioprospecting pipeline. Slow-growing bacteria are to be cultivated using novel isolation chip technology *in situ*, and classified using NGS. Compounds showing significant bioactivity will be subjected to characterization by MS, purification by HPLC, structural elucidation by NMR, and evaluated *in vivo* for toxicity using a zebrafish-model.