Seconda Università di Napoli

PhD Program in Molecular Life Sciences Abstract Book PhDays - Caserta Sept 26-27, 2016

Session 1. Structure and Function of Biomolecules

Isolation of novel bioactive proteins from the edible mushroom *Agrocybe aegerita*

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The edible *Agrocybe aegerita* mushroom (known as Pioppino) is a Basidiomycete belonging to the order Agaricales. It is greatly appreciated for its taste and flavour and highly known in the Campania region (particularly in Aversa territory) because it commonly grows on old poplar trees, used as support in grapevines cultivation. In this framework, during a screening of edible wild local species, our research group has started to study Pioppino mushroom to investigate its bio-chemical composition, nutritional values and antioxidant capability[1]. On the other hand, Pioppino mushroom is well known to be rich in several organic metabolites with biological activities[2] that could explain its use in traditional medicine[3]. *Vice versa,* few information is available in literature about proteins/enzymes with possible biotechnological and biomedical applications, as richly reported for all other mushrooms.
Therefore, with the aim of researching the presence of bioactive proteins, we have started an investigation of enzymatic activities such as protease inhibitors, peroxidases and toxins that inhibit protein synthesis *in vitro* as ribosome inactivating proteins, RNases and ribotoxins.
Preliminary results show that protein extracts from Pioppino inhibit protein synthesis *in vitro* especially testing the basic protein pool obtained after partial purification with traditional chromatographic techniques.

Metal ion replacement in prokaryotic zinc-finger domains: Pb(II) as xenobiotic cofactor

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Zn(II) ion binding to a proteic domain is a principal event in the achievement of the correct fold in the classical zinc finger domain since the motif is mainly unfolded in the absence of the metal. However, in the case of prokaryotic zinc finger the bigger ββββαα domain shows a larger hydrophobic core that plays a major effect in the folding mechanism. For these reasons, following the great attention devoted to unveil the effect of a xenobiotic 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 lead interaction with metallo-proteins.
We explore the consequences of Pb(II) to Zn(II) substitution in Ros87, the DNA binding domain of the prokaryotic zinc finger protein Ros. We demonstrate how the lead ion is not capable to fully surrogate the folding role of the zinc ion in the protein, leading to only a small portion of folded protein with structural perturbations that result in a highly reduced capability to bind the DNA target. Our data both integrate and complement the information collected in the last few years about the cellular biology of lead and its structural and functional effects in order to contribute to a better comprehension of the Pb(II) well known toxicity in biological systems.

Using mass spectrometry for protein structural and functional studies: studying the Gadd45β/MKK7 complex, a new target in Multiple Myeloma.

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Gadd45β/MKK7 complex plays a key role in the onset of Multiple Myeloma (MM) in individuals expressing high levels of Gadd45β[1]. Gadd45β supports cell growth by suppressing the MKK7 kinase activity, thus compounds disrupting the complex selectively restore cell death providing an option for curing MM[1]. Recently, an inhibitor named DTP3 has been identified and deeply characterized *in vitro* and *in vivo*[1]. DTP3 is a D-tripeptide that prevents Gadd45β interaction with MKK7 by binding to the kinase. The site of DTP3 binding and the mechanism affording Gadd45β dissociation were still unknown. Using chemical crosslinking [2] and mass spectrometry approaches we have elucidated the sites of interaction between Gadd45β and MKK7 and between MKK7 and DTP3. 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. Data also evidence that Gadd45β and MKK7 sites involved in autoassociation. Interaction sites of MKK7 with DTP3 have been identified on kinase regions 113-136 and 259-274, which are spatially adjacent and form a shallow cavity close to the catalytic pocket. Data are consistent with a mechanism involving Gadd45β/MKK7 homo- and hetero-interactions which are impaired by the presence of DTP3.

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Alpha- and beta-cyclodextrin inclusion complexes with 5-fluorouracil: structural characterization and cytotoxic activity evaluation.

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Cyclodextrins (CDs) are natural macrocyclic oligosaccharides containing relatively hydrophobic central cavity and hydrophilic outer, able to form inclusion complexes with a wide variety of guest, affecting their physicochemical and pharmaceutical properties [1,2]. In order to obtain an improvement of the low solubility and bioavailability of 5-Fluorouracil (5-FU), a pyrimidine analogue, used as chemotherapeutic agent in the treatment of the colon, liver and stomac cancers [3], the drug was complexed with alpha (α-CD) and beta-cyclodextrin (β-CD) [4-6]. The inclusion complexes were prepared in the solid state by kneading method. The formation of the inclusion complexes were confirmed by FT-IR Spectroscopy, X-ray Powder Diffractometry and NMR Spectroscopy. In solution, the 1:1 stoichiometry was established by Job plot method for all the inclusion complexes [7]. As a consequence of the deprotonation at two different possible sites, N1 or N3, 5-FU can present different pKa values; for this reason the binding constants for the complexes were determined by UV-Vis titration at different pHs. Molecular docking studies were performed to deeply investigate interactions of cyclodextrins with guest compound in terms of structural description. Furthermore, the cytotoxic activities of 5-FU and its complexation products with α-CD and β-CD were evaluated using the MTT-assay on Caco-2, MCF-7, Hep G2 and A-549 cancer cell lines. Results obtained indicate that the formation of complexes can improve the properties of guest to better its bioactivity.

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

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Cripto performs key functions in embryonic development, stem-cell differentiation and tumorigenesis. It contains two functionally and structurally independent domains of about 40 residues: the EGF-like domain, which binds to Nodal, and the CFC domain, which binds to ALK4. Both domains are essential to promote tumor growth by activating the Smad2/3 intracellular signaling through the ALK4/Cripto/Nodal/ActRIIB receptor complex [1]. His120 and Trp123 within the CFC domain are crucial hot-spots for Cripto-ALK4 recognition [2]. To obtain soluble inhibitors of the Cripto-ALK4 interaction we have designed a set of new highly constrained bi-cyclic CFC-like peptides containing these two key residues in different conformations and with different exposure. Linear peptides are prepared by SPPS, cyclized around a tri-bromo-methylbenzene (TBMB) scaffold [3] and tested for binding to ALK4 by SPR assays. One bi-cyclic peptide, named B3, binds ALK4 with an affinity of about 200 nM, about 50 higher compared to that of the refolded CFC domain. To understand the mechanism of B3-ALK4 recognition and to improve the affinity, we have designed, prepared and tested a set of Ala-scan peptides. We have identified residues underlying the recognition and those that can be further optimized for obtaining more potent and efficient ALK4 binders.

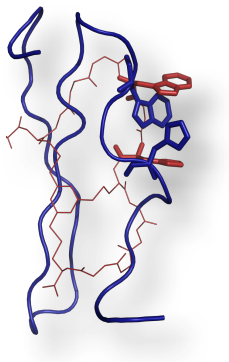

Fig. 3 NMR molecular model of hCFC *superimposed to the* model of B3 bi-cyclic structure

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De novo variant calling from RNA-seq in non-model species: a promise land

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The analysis of genomic variations is becoming an important part of clinical studies and is a fundamental part of population genetics, despite the cost reduction for sequencing these are still high, this is an obstacle for study of non-model species. Pooled RNA-seq could be a good compromise to study the population genetics of non-model species in a cost effective way, also for the species where a reference sequence is not available.
Here we present a pipeline for variant calling from RNAseq data, using RNAseq data of lympoblastoid cells lines form the GEUVADIS data set as a golden standard comparing it to 1000 genomes.

We downloaded ten random samples from gauvadis dataset repository and divided it in two pools to simulate pooled sequencing . Three random samples were used to de novo assemble a transcriptome with Trinity software. After a standard variant calling pipeline we made a comparison with 1000 genomes in terms of: i)position overlap ii) delta of frequencies iii) genotype concordance. The results for chromosome 22 show an average concordance of 30% with 1000 genomes dataset. The preliminary result shows a needing of more accurate filtering of the starting dataset taking into account several phenomenons happening in RNA-seq data.

Altered free D-aspartate, but not free D-serine, levels in the post-mortem brain of patients with schizophrenia

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A large bulk of evidence highlights the implication of N-methyl-D-aspartate receptors (NMDARs) hypofunction in the pathophysiology of schizophrenia. Given their role in the stimulation of NMDAR-dependent transmission, different studies are now suggesting an involvement of the endogenous D-amino acids, D-serine and D-aspartate, in schizophrenia. Herein, we evaluated the content of free D-serine and D-aspartate in the post-mortem hippocampus and frontal cortex (FC) of patients with schizophrenia. Moreover, in the same brain samples, we analysed both the expression of *D-aspartate oxidase* (*DDO*) mRNA and the enzymatic activity of DDO, the only known enzyme able to degrade endogenous D-aspartate. Interestingly, we found that free D-aspartate content, but not D-serine, is decreased in the FC of patients. However, we failed to find any difference in the expression of *DDO* mRNA between patients and controls, both in the FC and hippocampus. In contrast, in the FC, we revealed that the decrease in free D-aspartate content found in schizophrenia subjects is associated with increased DDO activity. These findings represent the starting point to investigate whether the altered D-aspartate metabolism found in the FC of patients may functionally contribute to the etiopathological events leading to schizophrenia.

NMR characterization and antiproliferative activity of secondary metabolites from Fabaceae species

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This year three plant extracts (*Astragalus boeticus*, *Ononis diffusa* and *Trigonella corniculata)* belonging to the Fabaceae family have been selected for their cytotoxic activity against colon cancer cell lines, including those resistant to conventional drugs. Thanks to 1D and 2D NMR experiments, the metabolic profiles of the extracts have been fully characterized and the putative compounds responsible for the antiproliferative properties have been identified in the mixture. Based on the preliminary data, attempting to isolate the cytotoxins from bioactive extracts, a focused phytochemical study has been carried out, using bioguided-fractionation techniques. Results from these experiments allowed to isolate and characterize, in a very short term, a cycloartane glycoside from *A. boeticus* and steroidal saponins from *T. corniculata.* Furthermore, the extracts' cytotoxicity on Cetuximab-resistant colon cancer cells have been proved.

The next goals will be the characterization of the active metabolites from *O. diffusa* extract and the setting up of further biological assays. Moreover, in order to better understand the mechanism of action of the active compounds, the Magnetic Resonance Spectroscopy will be used as tool to detect the metabolomic fingerprint of colon cancer cells in response to antineoplastic treatment.

Neuro-nutraceutical potential of Thyme and Sage Phenol-enriched Extracts

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As plant polyphenolscould be a double-edged sword for human brain, the main goal of the PhD research activity was to evaluate the neuro-bioactivity of newly prepared phenol complexesfrom *Thymus vulgaris* and *Salvia officinalis*, two medicinal and aromatic plants with ancient use as culinary spices and herbal remedies. Dried leaves of theplantsunderwent UAM extraction using solvents with different polarity ($CHCl_3$ and MeOH). The alcoholic extractswere further fractionated to achieve a thyme (TPE) and a sage phenol-enriched extract (SoA541), whichwere metabolically profiled through LC-ESI-MS/MS techniques.Cytotoxic and oxidant properties of both the extracts were evaluatedtowards neuronal cell lines. TPE, consisting in sixteen polyphenols, exerted cytotoxic effect at doses of 62.5 and 125 μg/mL being able to increase intracellular ROS levels and to activate caspases.SoA541, which accounted for ~ 50% of abietanediterpenes and ~ 40% of phenylpropanoid constituents, exhibited antioxidant effectiveness at dose levels <125 μg/mL and inhibited AChE enzyme to a far greater extent than donepezil.The promising results suggest the need for further research to improve our data and investigations into the potential use of these natural products as neuro-nutraceuticalremedies to counteract brain diseases using effective preclinical and clinical investigation tools.

Structural and dynamic properties of the Hepatitis C Virus proteins: E1 and NS5A proteins

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Hepatitis C virus (HCV) is one of the most prevalent serious infection in the world. To date the prohibitive costs of antiviral treatments limit access to therapy and no vaccine is available. During the first two years of the Ph.D. course my activities were focused on the structural/dynamic properties of the E2 proteins (1, 2, 3). In the third year, the activities have been extended to two other HCV proteins: the envelope protein E1 and the non-structural protein denoted as NS5A. Molecular dynamics simulations (MD) carried out on HCV E1 have highlighted the structural properties of the protein region specifically recognized by monoclonal antibodies. Interestingly, simulations carried out on different oligomeric forms of the protein have shown that the E1 monomer may be stable in solution, although crystallographic studies suggested that the protein assumes a dimeric structure (4). As anticipated above MD studies were also extended to a portion of NS5A, a key factor in the HCV genome replication, which is an Intrinsically Disordered. To gain insights into the conformational ensemble of this fragment an innovative protocol of Replica Exchange MD that was based on the use of the KBFF potential. The REMD results indicate that this novel force field is able to reproduce some of the features experimentally assessed for this peptide (5). This observation suggests that the conformational ensemble generated by these simulations is representative of the actual structural basin of the fragment.

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Unveiling the cellular role of DDX11, a Fe-S cluster DNA helicase, involved in genome maintenance

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DDX11 is a Fe-S cluster-containing Super-Family 2 DNA helicase, genetically linked the chromosomal instability disorder Warsaw breakage syndrome.
I present evidence that DDX11 establishes a physical and functional interaction with human Tim, a conserved protein forming together with Tipin a hetero-dimeric complex, the *Fork Protection Complex* (FPC) implicated in S phase checkpoint and replication fork progression.
I found that Tim stimulates DDX11 DNA binding and helicase activity on substrates that mimic key intermediates of DNA replication/repair/recombination processes, such as G-quadruplex and D-loop containing DNA molecules. Surface plasmon resonance measurements indicate that DDX11 directly interacts with Tim. DNA fiber track assays with HeLa cells exposed to hydroxyurea demonstrated that Tim or DDX11 depletion significantly reduced replication fork progression compared to control cells; whereas no additive effect was observed by co-depletion of both proteins. Moreover, Tim and DDX11 are epistatic in promoting efficient resumption of stalled DNA replication forks in hydroxyurea-treated cells. This is consistent with the finding that association of the two endogenous proteins in the cell extract chromatin fraction is considerably increased following hydroxyurea exposure. Overall, our studies provide evidence that Tim and DDX11 physically and functionally interact and act in concert to preserve replication fork progression in perturbed conditions.

NMR assignement of the DNA binding domain of Ml1 protein from *Mesorhizobium loti*.

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Affiliation:

The DNA recognition mechanism by proteins play a crucial role in regulatory process that controls the flow of genetic information. The first prokaryotic Cys_2His_2 zinc finger domain has been identified in the transcriptional regulator Ros from *Agrobacterium tumefaciens*. The NMR structure of the minimal DNA binding domain of Ros (named Ros87) indicates that the protein contains a globular domain in which the zinc ion is tethraedrally coordinated by two cysteine (Cys24, Cys27) and two histidines (His37, His42). Recently more 300 Ros homologues have been identified in a large number of bacteria with a sequence identity beetween 35% and 80% to the Ros protein. We have considered one of five homologues protein from *Mesorhizobium loti*, Ml1. Ml1 protein is a zinc-lacking protein that doesn't contain the Cys_2His_2 motif like Ros. The first cysteine in the coordination sphere is replaced by an acid aspartic residue however it is able to bind the Ros DNA target sequence. The aim of my project is NMR structural characterization of the Ml1 protein DNA binding domain (residues 24-142).

The data obtained demonstrate how the prokaryotic zinc finger domain can exploite different combinations of coordinating aminoacids or fully overcome the structural metal cofactor requirement to properly fold and function.

The human Paraoxonase 2: biochemical and functional characterization

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This research project is focused on the human Paraoxonase 2 (PON2), whose main activities are: a calcium-dependent hydrolytic activity mainly on lactones and a redox function that reduces the levels of ROS (reactive oxygen species). This research project is focused on an engineered form of PON2, obtained by removing the highly hydrophobic N-terminal region and by the insertion of six amino acidic substitutions which stabilize the structure. The rPON2 has been over-expressed in *E.coli*, refolded *in vitro* from inclusion bodies and purified, yielding an enzyme with the same characteristics of the full length enzyme. The biochemical characterization confirmed the primary activity on 3OC12-HSL, an homoserine lactone produced by Pseudomonas aeruginosa (PAO1). According to that were performed *in vitro* experiments of inhibition of the biofilm formed by Pseudomonas aeruginosa (PAO1) which have confirmed that rPON2 is more effective than PON1. Surprisingly rPON2 has also showed small but significant activity against organophosphorothiotes pesticides, m-parathion, coumaphos and malathion. Recent studies have revealed that the activity of PON2 is subjected to a mechanism of inactivation fast and reversible, consistent with a post-translational modification. The study of the post-translational modifications has revealed that Lys 168 is ubiquitinated and this modification affects the catalytic activity of the protein. Finally we have also expressed and characterized 3 mutants of PON2: a deleted isoform of PON2 (del 123-134 rPON2), Ser311Cys rPON2 and Gly148Ala rPON2, because these polymorphism and isoforms have a physiological relevance.

Insights into the structural and functional features of the tumor associated protein hCA IX

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Human Carbonic anhydrase IX (hCA IX) is a protein belonging to the hCA family,[1] which is highly overexpressed in tumors and involved in their survival and metastatic spread.[2]
Recent studies demonstrated that hCA IX interacts with CAND1, a nuclear protein involved in gene transcription and assembly of SCF ubiquitin ligase complexes.[3,4] Since lower hCA IX levels have been observed in cells with CAND1 suppressed by shRNA-mediated interference, it has been suggested that this interaction could be necessary for CA IX stabilization. It has been also demonstrated that the CA IX region required for interaction with CAND1 is the C-terminal one (Leu418-Ala459).[3]
During my Ph.D, the molecular determinants responsible for the CA IX/CAND1 interaction have been identified by means of a multidisciplinary approach. In particular, firstly CAND1 has been cloned and expressed in *E. coli*. At the same time, peptides of different length, mimicking the CA IX C-terminal region have been synthesized. Protein-peptide binding assays by means of Surface Plasmon Resonance and fluorescence spectroscopy have been then set up to confirm the occurrence of a direct CA IX/CAND1 interaction. Subsequently the CA IX/CAND1 complex has been modelled by using a docking approach and validated by means of site-directed mutagenesis.

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Session 2. Human Genetics

Disclosing the molecular mechanism underlying Early Onset Paget's Disease of bone.

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Paget's disease of bone (PDB) is a late onset disorder (>50 years), characterized by focal increases in bone turnover, primarily caused by dysregulation of osteoclast bone resorption, a mechanism typically regulated by an F-actin–rich structure, called sealing zone. Mutations in the *SQSTM1* gene, encoding the p62 protein, are the most common cause of PDB. However, the genetic landscape of Paget's disease is not fully elucidated.
We applied whole-exome sequencing on a family with 11 members affected by severe and early onset PDB (mean age at diagnosis 27,3±5,8 yrs; mean number of affected skeletal sites 5.8± 2), and identified 4 heterozygous changes in 4 candidate genes (*CAMTA2*, *GGT6*, *MYH3* and *PFN1*), all mapping to 17p13 chromosome region. Among them, the *PFN1* gene appeared to be the responsible gene, given that the identified mutation is a deletion, resulting in premature termination of the profilin-1, involved in actin polymerization. We demonstrated that the mutant *PFN1* mRNA doesn't undergo nonsense-mediated decay and showed that the truncated profilin-1 is partially degraded by the proteasome. Moreover, cells expressing the PDB-causing *PFN1* mutation form cytoplasmic aggregates. Further studies are on-going to disclose the fate of the mutant.

Alzheimer's Disease and allelic variants in late onset forms: focusing on CD33 and TREM2.

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Background. Genome-wide association studies (GWAS) identified variations in over 20 loci associated with disease risk of Alzheimer's disease (AD). Three SNPs identifying missense variants in microglial receptors CD33 and TREM2 were described to be associated with the risk of late-onset Alzheimer's disease (LOAD). In particular, the SNPs rs3856444 and rs12459419 in CD33 were found to confer either strong protection or elevated risk of LOAD. We collected 253 Caucasians (145 women [57.3 %] and 108 men [42.7%] mean age 60 – 85 years) ascertained with AD and start analyzing them for the presence of these variants.
Methods. Genomic DNA was extracted from WBC and analyzed by HRM method. We used Primer3 to design PCR primers and Sanger method to sequence reference control samples and confirm our analyses.
Results. Our patients, show the coinheritance of rs12459419 major and minor alleles with rs3865444 major and minor alleles in 52 heterozygous individuals. Homozygous samples have to be spiked with reference DNA to better distinguish homozygous mutations from wild-types.
Conclusions. The rs3865444 SNP, located in the promoter region, and the rs12459419 SNP, localized in the exon 2, seem to work in concert in the modulation of the disease progression. Further investigations are in progress.

Molecular mechanisms of pathogenic mutations in Frontotemporal dementia

PhD Student 31st cycle: Sabrina Napoletano
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INTRODUCTION

Frontotemporal dementia (FTD) is a neurodegenerative disorder characterized by clinical and neuropathological heterogeneity and high genetic complexity.

Granulin gene (*GRN*) is a major genetic determinant and Progranulin protein (PGRN) haploinsufficiency is the proposed disease mechanism. PGRN is a secreted glycoprotein with several roles and expressed across a wide range of tissues including the brain. How PGRN deficiency can lead to brain dysfunction is not yet identified.

OBJECTIVES

Identify and functionally analyze *GRN* mutations causing FTD phenotype. Study molecular pathways affected by PGRN deficiency.

METHODS

We collected and screened WBC from FTD patients and controls matched for age, sex and geographic regions.

We identified and analyzed the effect of *GRN* mutations at transcriptional and translational levels.

Generation of an in vitro cell model of PGRN deficiency using CRISPR-Cas9 for *GRN* knock-out.

RESULTS

We identified *GRN* exon six deletion *g10325_10331delCTGCTGT* in three FTD familial cases. This mutation was previously described in two sporadic cases but was never associated with familial cases. Transcriptomic and proteomic analysis revealed a decrease of PGRN levels in patients carrying the mutation.

CONCLUSIONS

These findings provide further support for a previously proposed role for *GRN* in the genetic etiology of FTD and its phenotypic variability.

Identification and characterization of molecular defects causing the Beckwith-Wiedemann syndrome

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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 90 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.
Furthermore, we are investigating the possible correlation between the severity of the ICRs epimutations (% of methylation) and the presence of specific clinical signs. Preliminary data show that low levels of ICR2 methylation correlate to a major risk of omphalocele and, intriguingly, could be indicative of a common defect between BWS and Long QT syndrome (a cardiac condition due a delayed repolarization of the heart).

Finding new connections in the transcriptional regulation of Lysine-specific demethylase 5C (KDM5C), a disease gene involved in Neurodevelopmental disorders (NDDs)

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Goal of my PhD project is to dissect the complexity of the transcriptional regulation of Lysine-specific demethylase 5C (KDM5C), an X-linked gene involved in X-linked Intellectual disabilities (XLID) and Epilepsy. We analysed three NDD proteins able to stimulate *KDM5C* transcription: PHD Finger Protein 8 **(**PHF8), a H3K9 demethylase, and the two transcription factors Zinc Finger Protein 711 (ZNF711) and Aristaless related-homeobox (ARX). We identified the regions on *KDM5C* promoter required for ZNF711 and PHF8 activity, proving that both proteins co-occupy a region located near the TSS. We also established a cooperative activity of ZNF711/PHF8 and proved that PHF8 interacts with ARX. Thus, we analysed the functional impact of XLID/Epilepsy mutations affecting *ZNF711*, *PHF8* and *ARX* genes proving that they severely alter the functioning of KDM5C-H3K4me3 path. Finally, we screened a number of epi-compounds to correct KDM5C-H3K4me3 fault. As cell disease model, it has been used the neuronally-differentiated *Arx* KO/*Kdm5C*-depleted ES cells presenting GABAergic abnormalities and a global H3K4me3 increase. By using the HDAC inhibitor Suberoyl Hydroxamin Acid (SAHA), a rescue of KDM5C-H3K4me3 damage has been obtained in treated-neurons. Ongoing *in vivo* efforts will allow us to explore potential epi-strategies to treat NDDs caused by defects in KDM5C path.

A novel extracellular matrix multisystem syndrome due to a dominant mutation in LAMA5 gene: implication for ECM functioning and remodeling and its interplay with other connective proteins

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We report a clinical description as well as genetic and functional studies of a novel dominant connective syndrome characterized by laxity of the visceral ligaments, impaired wound healing, serum negative arthritis, mild alopecia and teeth defects. Most of the patients exhibit severe myopia associated with retinal detachment and night blindness. This combination of clinical signs has never been described before, making unique this family.
Through whole exome sequencing we discovered the p.V3140M mutation in *LAMA5*gene responsible for syndromic traits, while p.K383E mutation in *P4HA2* is associated with the severe myopia. The P4HA2 mutation causes a decrease of expression of both P4HA2 RNA and protein and impairs the collagen deposition. The laminin mutation, located in the LG3 domain, crucial for protein folding and for pathways induction through integrin's binding, alters the amount of cleavage peptides, perturbs the SHH-GLI1 pathway, which is up regulated in patients, while the ECM proteins are strongly inhibited, suggesting a condition resembling fibrosis.
Human skin biopsies showed dermal papilla alteration, a germinative layer reduction and an early arrest of hair follicle down growth, mainly observed in the patients carrying both mutations. A similar defect is observed in the Lama5 knock-in mouse model generated in our laboratory.

Targeted Next Generation Sequencing strategies for genetic heterogeneous disorders

PhD Student 29 th cycle: Giuseppina Di Fruscio
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Next Generation Sequencing (NGS) technologies are now the "gold standard" to identify small mutations in highly heterogeneous disorders.
We have designed several different targeted NGS platforms to analyse some of these conditions:
MotorPlex allows us to analyse 90 genes involved in neuromuscular disorders and to find causative mutations in 43% of patients. With *NephroPlex,* we were able to test 115 disease genes causing a renal phenotype, including *PKD1* that hampered the routine analysis because of the presence of six pseudogenes. *NephroPlex* identified causative variants in 83% of samples. *LysoPlex* allows us to clarify the role of lysosomal-endocytic-autophagic pathway, analysing 891 genes; in particular, we included 89 genes associated to lysosomal storage disorders. We have identified disease-causing mutations in 63% of patients. With *RetPlex*, we were able to clarify molecular causes of visual impairment of genetic origin, identifying causative variations in 140 genes involved in inherited retinal dystrophies. We identified causative mutations in 60% of patients.
In conclusion, NGS panels are a cheap and fast approach to study all the conditions characterized by genetic heterogeneity. In fact, it allows us to analyse a large set of genes in a huge cohort of samples in a relatively short times and at lower costs than WES or WGS.

Molecular, biochemical and histological characterization of Giant Cell Tumor arising on Paget's disease of Bone

PhD Student 29 th cycle: Giuseppina Divisato
Tutor: : Dr. Fernando Gianfrancesco
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Paget's disease of bone (PDB) is characterized by abnormalities of bone remodeling that rarely undergo neoplastic transformation, resulting in giant cell tumor of bone (GCT/PDB). Applying whole-exome sequencing in a family with 14 PDB-affected members, four of whom developed GCT, we identified p.Pro937Arg mutation in the *ZNF687* gene as responsible for GCT/PDB phenotype. Unrelated GCT/PDB individuals showed the same mutation, unravelling a founder effect. Functional studies disclosed the role of *ZNF687* in bone biology and they revealed its up-regulation in GCT/PDB tumor tissues and in the peripheral blood of PDB patients with different genetic background. ChIP-seq analyses showed that *ZNF687* is a NFkB target gene (iper-activated in PDB), justifying its up-regulation in PDB and highlighting its potential role as PDB-risk biomarker. In addition, we also excluded a *two hit* in *H3F3A* (responsible for conventional GCT) as a trigger event for GCT/PDB development. Accordingly, we also demostrated that the different genetic signature of GCT/PDB correlates with an alternative biochemical and histological profile of the tumor compared with conventional GCT. In conclusion, our findings reveal that the peculiar clinical features of GCT/PDB are associated with a certain genetic profile, resulting in a specific biochemical and histological behaviour of the tumour.

Genetic interactions among Fanconi Anaemia repair genes

PhD Student 29 th cycle: Marcello Germoglio
Tutor: : Dr. Adele Adamo
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Fanconi Anaemia (FA) is a cancer predisposition syndrome, also inducing sterility and developmental defects. The nematode *C. elegans* has emerged as a powerful system for the study of Fanconi Anaemia in the context of a whole organism. Similar to their vertebrate counterparts, mutants in *C. elegans* FA pathway genes present a significant increase in developmental defects, hypersensitivity to inter-strand cross-linking (ICL) agents and display chromosomal aberrations and enhanced mutagenesis following treatment with these agents (Collis et al. 2006; Youds et al. 2008).
The key FA *fcd-2* gene is involved in double-strand breaks repair using homologous recombination and in preventing the careless repair by non-homologous-end-joining (NEHJ; Adamo *et al.* 2010).
My results leads to new insights in the role, localization and interactions of the FCD-2 protein, in somatic and germ-line. Furthermore, we identified and isolated a new spontaneous recessive mutant, called clt-2, in fcd-2 mutant background. clt-2 restores the NHEJ enhanced regulation of fcd-2 mutant and rescue the hypersensitivity to ICL-inducing agents and developmental defects frequency of fcd-2 mutant to the wild type level. Deepening the *clt-2* genetic interactions among FA repair genes will help us to better understand the role of FCD-2 in NHEJ regulation.

Session 3. Molecular Cell Biology

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

PhD Student 31st cycle: Emilia Pascale
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The Ultraconserved Elements (UCEs) are an emerging group of genomic sequences, showing the peculiar feature to retain extended perfect sequence identity between the human, mouse, and rat genomes. Moreover, it has been shown that a large fraction of UCEs is transcribed (T-UCE) but their function is largely unknown in physiological context, for instance in stem cell biology. By genome-wide expression profiling, we identified 43 T-UCEs de-regulated during ESC differentiation and, among them we focused our studies on uc.170. We first assessed the exact length of the transcript containing uc.170 by performing cDNA-walking experiments followed by RACE-PCR, and we named the new transcript as *T-UCstem1*. In order to investigate the functional role of T-UCstem1 in ESCs, we generated T-UCstem1 knockdown (KD) ESC clones and we analysed their molecular and cellular features. T-UCstem1 KD ESCs showed cell cycle arrest and partly undergo to apoptosis. Moreover, KD ESCs showed an unusual morphology that prompted us to perform further phenotypical analysis. Interestingly, despite their morphology and altered proliferation rate, we demonstrated that KD ESCs retained a proper expression of pluripotency markers. To get mechanistic insights, we analysed RNA-seq profiling of KD and Control ESCs, highlighting a peculiar molecular signature in ESCs upon T-UCstem1 silencing.

Unraveling the inflammatory cells contribution of Cripto to skeletal muscle regeneration

PhD Student 31st cycle: Francescopaolo Iavarone
Tutor: : Dott.ssa Gabriella Minchiotti
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Skeletal muscle tissue responses following injury and disease are highly coordinated processes involving the interactions of different cell types among which the muscle stem cells and the inflammatory cells. How inflammatory and myogenic components integrate to coordinate skeletal muscle regeneration still remains unknown. Our recent data point to a key role of the developmental factor Cripto, which is re-expressed in response to muscle injury both in myogenic cells and in a subset of macrophages (MPs). Cripto is required in the satellite cells to achieve effective muscle regeneration *in vivo*, while, to date, the role in the inflammatory cells still remains unknown. To further dissect the Cripto's contribution in the inflammatory cells, we developed a mouse model based on the transplant of Cripto KO haematopoietic progenitors (Cripto H-LOF) and analyzed Cripto KO muscle-infiltrating macrophages after acute injury. Preliminary data showed altered distribution of different MPs population in Cripto H-LOF compared to control. Moreover, RNA-Seq analysis of infiltrated cd11b(+) cells showed a significant upregulation of a large set of extracellular matrix genes, suggesting that Cripto KO MPs undergo a mesenchymal-like transition. To further characterize the effect of Cripto H-LOF on muscle regeneration we generated macrophage-specific Cripto KO mice. The molecular and functional characterization of this new genetic model will help clarifying the role of Cripto in the inflammation that occurs during the muscle repair.

Secretory phospholipase A_2 role in osteoclastogenesis

PhD Student 31st cycle: Maria Mangini
Tutor: : Dott.ssa Stefania Mariggiò
Affiliation: Institute of Protein Biochemistry, CNR

Secretory phosholipases A_2 ($sPLA_2$) are lipolytic enzymes that cleave glycerophospholipids at the *sn-2* position, which releases a free fatty acid and a lysophosholipid (1). $sPLA_2$ are involved in several cellular processes, from inflammation to cancer, and under conditions that require cell-to-cell fusion (1). We are pursuing the elucidation of the mechanism of action of $sPLA_2$-IIA in the osteoclastogenesis process, to unravel its involvement in mature osteoclast syncytium formation. Data in the literature have demonstrated that $sPLA_2$-IIA expression is induced under conditions characterised by high bone-resorption activity (2), and that $sPLA_2$-IIA inhibition prevents bone loss after ovariectomy in mice (3). Moreover, we have found that $sPLA_2$-IIA inhibition impairs *in-vitro* osteoclast differentiation of precursor cells, which reduces expression levels of differentiation markers and blocks cell-to-cell fusion. However, the precise role of $sPLA_2$-IIA in osteoclastogenesis remains unknown, as well as its mechanism of action, which might require involvement of its lipid metabolites or $sPLA_2$-IIA interactions with other proteins in a catalysis-independent manner (1,4). Changes in cell-lipid composition will be monitored by comparative lipidomic analysis of precursor cells differentiated in the presence of $sPLA_2$-IIA inhibitors, and of multinucleated mature osteoclasts. In the same cell systems, detailed evaluation of the $sPLA_2$-IIA interactome will be carried out by enzyme immunoprecipitation, through application of the SILAC methodology. These two approaches will shed new light on the osteoclast differentiation process and the $sPLA_2$-IIA mechanism of action, to provide a framework to propose $sPLA_2$-IIA as a drug target in pathologies with exacerbated osteoclast activity.

Antiproliferative activity of miR-125a toward human hepatocellular carcinoma cells

PhD Student 31st cycle: Panella Marta
Tutor: : Prof. Aniello Russo
Affiliation: Di.S.T.A.Bi.F. Seconda Università degli Studi di Napoli.

Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide. MicroRNAs (miRNAs) play crucial roles in cancer development and progression. My PhD project is aimed at the investigation of the tumor suppressor role of miR-125a in HCC. Firstly, the antiproliferative activity of miR-125a was demonstrated. In particular, upregulation of the microRNA inhibited proliferation of HCC cells by p21/p27-dependent cell cycle arrest in G1. Then, a number of miR-125a validated target transcripts relevant to the antiproliferative activity in different cell lines were analyzed. Among these targets, Sirtuin 7, matrix metalloproteinase 11 and Zbtb7a proto-oncogene resulted downregulated upon cell transfection with miR-125a mimic. In addition, a novel miR-125a target, c-Raf, was validated, supporting its contribution to the antiproliferative activity of the miRNA. Interestingly, miR-125a was also found to be involved in the mechanism of action of sorafenib, an antitumor drug for advanced HCC, since miR-125a expression was induced by the drug and a miR-125a inhibitor counteracted the antiproliferative activity of sorafenib. Encouraged by these results, the expression of miR-125a and that of its targets were analyzed in HCC biopsies and in matched adjacent non-tumor liver tissues. Consistently with data obtained on cultured cells, miR-125a was found to be underexpressed in tumor tissues of most patients whereas its oncogenic targets were upregulated. Overall, these results support a tumor suppressor role for miR-125a and provide the basis for further investigations aimed at the characterization of the molecular mechanisms governing its expression.

Evaluation of adiponectin profile in Common Variable Immunodeficiency patients.

PhD Student 31st cycle: Rita Polito
Tutor: : Prof.ssa Aurora Daniele
Affiliation: Di.S.T.A.Bi.F. Seconda Università degli Studi di Napoli.

In my PhD project, adiponectin expression and its oligomeric distribution have been investigated in patients affected by Common Variable Immunodeficiency. CVID is characterized by hypogammaglobulinaemia. Adipose tissue is strongly associated with development and progression of immune disorders through adipokine secretion. Adiponectin has beneficial properties and is involved in inflammation and immunity processes. Adiponectin circulates as oligomers of different molecular weight: HMW, MMW and LMW. The HMW are the most biologically active oligomers.
In this study, we are analyzing adiponectin profile by Elisa, Western Blotting and FPLC assay in CVID patients and age- and sex-matched controls. Our preliminary results show that total adiponectin is decreased in CVID patients compared to controls. In addition, Western blot demonstrated presence of HMW, MMW, and LMW oligomers in both controls and CVID patients but the densitometric evaluation of adiponectin oligomers showed a specific decrease of HMW oligomers in CVID patients. In conclusion, our findings support the hypothesis of a biological role of adiponectin in immune disorders and therefore in CVID. The molecular mechanisms through which adiponectin levels are down-regulated in immune system are not clearly defined, but the down-regulation of adiponectin and its HMW oligomers may contribute to the establishment of the inflammatory typical of CVID.

Regulation of PPARγ signaling through alternative splicing and dominant negative isoforms

PhD Student 31st cycle: Simona Cataldi
Tutor: : Prof. Alfredo Ciccodicola
Affiliation: Istitute of Genetics and Biophysics "Adriano Buzzati-Traverso", CNR, Naples.

PPARγ is a transcription factor that drives the expression of genes crucial in adipogenesis, glucose and lipid metabolism. Loss-of-function mutations in *PPARG* gene have been associated with insulin resistance, obesity and an increasing risk to develop type 2 diabetes (T2DM). Most of the mutations fall in the ligand-binding domain (LBD) and determine the translation of dominant negative isoforms. During the first year of my PhD, I focused on the study of a new PPARγ isoform, PPARγΔ_{LBD}, generate by alternative splicing. Similar to mutant receptors, it lacks the LBD acting as dominant negative. The results of my project show that the ligand-mediated PPARγ activation induces the splicing of *PPARG* pre-mRNA, leading to an increased expression of PPARγΔ_{LBD}. Moreover, I observed that PPARγ activation induces also the expression of some splicing factors, including ASF/SF2. Interestingly, knockdown of this factor significantly prevents the PPARγΔ_{LBD} expression, indicating that ASF/SF2 is involved in alternative splicing of *PPARG* gene. It indicates a new mechanism in PPARγ regulation. Thus, given the crucial role of this transcription factor in metabolic homeostasis, the 2nd year of my PhD will be devoted to definitely address its relevance both in physiological and pathological conditions, such as in insulin resistance and T2DM.

Control Systems of the secretory pathway

PhD Student 30 th cycle: Alessandra Varavallo
Tutor : Dr. Alberto Luini
Affiliation: Institute of Protein Biochemistry, CNR, Napoli

Secretory pathway is constituted by a series of steps useful to move proteins inside and outside of the cell. In order to keep homeostasis these membrane fluxes need to be balanced. Since our previous description of signaling circuits regulating retrograde and anterograde traffic by KDEL receptor/Src/PKA machinery, we propose that the secretory pathway might be regulated in a control system fashion. Control system devices are usually represented by signaling cascades actuated as cellular response to external perturbation. According to this theory, we decided to generate external perturbations in order to investigate signaling circuits maintaining cell homeostasis during protein secretion. Using antibody microarray and proteomic data analysis we revealed that during Endoplasmic Reticulum (ER) cargo export, the regulatory Protein Kinase A(PKA) RII subunit was phosphorylated on Ser99 and the A-kinase anchor *protein* (AKAP) -KL was phosphorylated by PKA and besides we found new possible G Protein-Coupled Receptor Class C Group 5 Member A (GPRC5a) interactors that could be involved in the signal transductions activated at Trans Golgi Network. Moreover, in order to reconstruct still unknown signaling cascades, we started to collect in a database all relevant information on signaling molecules. In this way, following the compartmentalization and pathways involved, we will be able to draw unknown protein networks and to build up a new interactive tool for molecular biology as well as interactome studies, useful to follow protein signaling cascade thought different organelles.

Insights into neuroectoderm and mesoderm cell lineage segregation during early vertebrate development from *Cripto* gene

PhD Student 30 th cycle: Sara Mancinelli
Tutor : Dr. Giovanna L. Liguori
Affiliation: Institute of Genetics and Biophysics ABT - CNR Naples

The relation existing between mesoderm and neuroectoderm is highly dynamic during the different stages of embryo development. First, the mesodermal and neural fates have to segregate while later on, mesodermal structures are fundamental for proper neural plate induction, maintenance and regionalization. The puzzle becomes very complex if we consider that the same signalling pathways (Tgf-, Wnt and FGF) are involved in both neural and mesoderm induction. *Cripto* gene, a key component of the majority of these pathways, is expressed in the forming mesoderm, being downregulated in the external ectoderm, included neuroectoderm.
The current work aims to unravel the mechanisms underpinning cell lineage separation between mesoderm and neuroectoderm, by means of *Cripto* gain of function experiments in neural progenitors. The phenotypic analysis of *Cripto* overexpressing neural progenitors shows a strong inhibition of neural differentiation and suggests transdifferentiation towards mesenchymal fate, pointing to a role of the mesoderm gene *Cripto* in redirecting the neural fate towards mesoderm. *In vivo* analysis is also ongoing to confirm these data. The study will contribute to unravel the mechanisms underpinning cell determination and lineage segregation and give new insights into neural development and diseases.

CtBP1-S/BARS regulates Lipid Droplet biogenesis

PhD Student 29 th cycle: Angela Filograna
Tutor : Daniela Corda
Affiliation: Institute of Protein Biochemistry, CNR, Naples

Lipid droplets (LDs) are intracellular lipid-storing organelles surrounded by a phospholipid monolayer. LDs emerge as metabolically active organelles involved in many biological processes and linked to the pathogenesis of several dysfunctions including metabolic diseases (obesity, diabetes, atherosclerosis, hepatic steatosis). The mechanism underlying LD formation is still poorly understood and the molecular players that take part in this process have to be identified.
Here, we provide evidence that a member of the C-terminal binding protein (CtBP) family, CtBP1-S/BARS (for brevity BARS), associates with LDs and is necessary for their formation. BARS is a fission-inducing protein that regulates several membrane trafficking events. Here, we show that BARS is highly expressed during the 3T3-L1 adipocyte cell differentiation. Interestingly, either the depletion or the inhibition of BARS strongly reduced the number of LDs and increased their size (as also shown in HeLa cells), indicating a central role of BARS in LD biogenesis.
Furthermore, we found that BARS interacts and activates Acyl-CoA:lysocardiolipin acyltransferase (LCLAT1/AGPAT8) that catalyses the acylation of lysophosphatidylinositol (LPI) to form phosphatidylinositol (PI), one of the main LD surface phospholipid components. Interestingly, we demonstrate that both BARS and AGPAT8 are recruited onto LDs where they interact and their specific LD membrane targeting is required for proper LD biogenesis. Our results shed new light on mechanisms underlying neutral lipid storage and highlight BARS and its binding partner AGPAT8 as key regulators in the biogenesis of lipid droplets.

Potential role of the mono-adp-ribosyltransferase parp12 in the regulation of intracellular membrane traffic

PhD Student 29 th cycle: Laura Schembri
Tutor : Dr. Daniela Corda
Affiliation: Institute of Protein Biochemistry, CNR, Naples

The poly(ADP-ribosyl)polymerase (PARP) family is a class of enzymes that regulates the function of target proteins by transferring ADP-ribose to specific residues, using NAD^+ as substrate. The cellular events regulated by these enzymes are diverse and range from transcriptional regulation to cell stress response, suggesting an important role for this post-translational modification in many cellular processes. PARP12 is a member of this family, mainly described to function in immune and stress response.
Here, we focused on PARP12 function under non-stressing conditions, analyzing the pathways in which this enzyme is involved through the identification of its substrates. We show that PARP12 – a mono-ADP-ribosyltransferase located at the *trans* side of the Golgi complex - contributes to maintain a correct Golgi morphology and modifies golgin-97, a Golgi-localized protein involved in the maintenance of Golgi architecture and in the regulation of intracellular traffic. Further, we demonstrated that PARP12 specifically regulates golgin-97 mediated trafficking routes, pointing at a potential role of this post-translational modification in the regulation of intracellular membrane traffic.

Organization of Golgi glycosylation reactions by matrix proteins

PhD Student 29 th cycle: La Prathyush Deepth Roy Pothukuchi
Tutor : Dr. Seetharaman Parashuraman
Affiliation: Institute of Protein Biochemistry, CNR, Naples

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 also present the progress in understanding the mechanism by which the matrix proteins may organize specific glycosylation reactions at the Golgi apparatus

Session 4. Gene regulation

Functional studies of a novel long non-coding RNA (MET-AS) in papillary thyroid carcinoma.

PhD Student 31st cycle: Daniela Esposito
Tutor : Dr. Valerio Costa
Affiliation: Institute of Genetics and Biophysics "Adriano Buzzati-Traverso", CNR, Naples.

Long non coding RNAs (lncRNAs) are transcripts >200 nt without protein coding capacity. They regulate the expression of protein coding genes and are involved in different cellular processes, such as cell growth and apoptosis, pluripotency and differentiation, and transformation through multiple mechanisms.
My PhD project focuses on a new lncRNA recently identified in our lab in patients with papillary thyroid carcinoma (PTC). It is transcribed antisense to *MET* oncogene, thus we named it *MET-AS*. Both genes are up-regulated in PTCs patients with $BRAF_{V600E}$ somatic mutation and *RET* gene rearrangements compared to other PTCs and control thyroids.
Preliminary data indicate that *MET-AS* is predominantly - if not exclusively - expressed in thyroid and that its knockdown in PTC cell lines impairs MET expression both at the mRNA and protein level, suggesting it is a new *MET* oncogene regulator. Moreover, its knockdown significantly reduces proliferation, migration and colony forming capability of thyroid cancer cell lines.
Other functional studies are still in progress to address how *MET-AS* regulates *MET* oncogene, and to confirm *MET-AS* involvement in PTC pathogenesis.

Age-Related Changes in D-Aspartate Oxidase Promoter Methylation Control Extracellular D-Aspartate Levels and Prevent Precocious Cell Death during Brain Aging

PhD Student 31st cycle: Daniela Punzo
Tutor : Dr. Alessandro Usiello
Affiliation: Seconda Università di Napoli (SUN), Ceinge (Na)

The endogenous NMDA receptor (NMDAR) agonist D-aspartate is degraded by the enzyme D-aspartate oxidase (DDO). D-aspartate and DDO display reciprocal occurrence. We show here that D-aspartate content in the mouse brain drastically decreases after birth while *Ddo* mRNA levels concomitantly increase. Interestingly, postnatal *Ddo* gene expression is paralleled by progressive demethylation within its putative promoter region. Consistent with an epigenetic control on *Ddo* expression, treatment with the DNA demethylating agent, azacitidine, causes increased mRNA levels in embryonic cortical neurons. In $Ddo^{-/-}$ mice, which show constitutively suppressed *Ddo* expression thus simulating persistent gene hypermethylation, we found substantially increased extracellular content of D-aspartate in the brain. In line with detrimental effects produced by NMDAR overstimulation, persistent D-aspartate elevation in $Ddo^{-/-}$ brains is associated with appearance of dystrophic microglia, precocious Caspase 3 activation and cell death. This evidence highlights the importance of *Ddo* demethylation in preventing neurodegeneration produced by non-physiological levels of D-aspartate.

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

PhD Student 31st cycle: Kumar Parijat Tripathi
Tutor : Dr. Mario Rosario Guarracino
Affiliation: Lab-GTP, ICAR-CNR

I am working as a capacity of research fellow in LAB-GTP on a theranostic project (therapeutic diagnostic) in LAB-GTP. From 2015-2016, I got enrolled in PhD program at Second University of Naples. The objective of my research work is to develop new methods, pipelines and software tools for the integration and analysis of high throughput transcriptomics data. In the first year of my PhD, I worked on several interesting projects in collaboration with IBP-CNR (Seetharaman Parashuraman) in developing novel method to compare the gene expression signature obtained from gene perturbation experiments, by using rank based non parametric statistical inference approach (*International Meeting on Computational Intelligence Methods for Bioinformatics and Biostatistics*, pp. 28-41. Springer International Publishing, 2016); secondly, we also develop 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, Daniela Evangelista and Mario Rosario Guarracino (A mixed integer programming-based global optimization framework for analyzing gene expression data. Manuscript under review in Journal of Global Optimization. Special edition for World congress of Global optimization Conference, Gainsfield, Florida, USA 2015). Apart from this, I also contributed substantially in developing novel tools to integrate and analyze functional genomics data such as Transcriptator (*PloS one* 10, no. 11 (2015): e0140268.) which is primarily a Python/JAVA based Computational pipeline to annotate assembled reads and identify non coding RNA from RNA-Seq data, and two data bases associated with the integration and annotation of non model organism *Hydra vulgaris* transcriptome (International Conference on Bioinformatics and Biomedical Engineering, pp. 355-362. Springer International Publishing, 2015., also accepted in special edition BMC bioinformatics 2016) and Skeletal muscle tissue specific transcripts of *Pan troglodytes* (International Meeting on Computational Intelligence Methods for Bioinformatics and Biostatistics, pp. 273-284. Springer International Publishing, 2016) respectively.

I also collaborated with Marcella Vacca from IGB-CNR, in the development of bioinformatics strategy to sort out non-neuronal cells variability from transcriptome profiling to understand the effects of Mecp2 loss of function in embryonic cortical neurons (*BMC bioinformatics* 17, no. 2 (2016): 189).

As a team member, I also contributed to the project of Reconstructing a Genetic Network from Gene Perturbations in Secretory Pathway of Cancer Cell Lines with Marina Piccirillo and presented the work in BMTL 2015, 19-21 October 2015 Napoli, and in BITS 2016, Salerno and along with IBP-CNR group, worked on the research project 'Dissecting the functions of the secretory pathway by transcriptional profiling' and presented the work **in** BMTL conference, 19-21 October 2015 Napoli.

Being a team member of LAB-GTP team along with my supervisor Mario Rosario Guarracino and Maurizio Giordano, I also took part in SBV-IMPROVER systox challenge, 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 write up for System toxicology computational challenge from organizer Phillip Morris international, Neuchâtel, Switzerland 19 may 2016. Currently, we are writing a manuscript for over arching publication in BMC Pharmacology and Toxicology.

At present, I am working on the development of novel pipeline, to detect and analyze the functional interpretation of non-coding RNA specific to circular in nature, using high throughput RNA-seq data. I am also working with my peers in collaboration with Dr. Alex Shu-Wing Ng, Harvard University on the development of novel integrated platform for the detection and analysis of the alternative splicing patterns, specifically related to the chimeric transcripts in Ovarian Cancer, using high throughput total RNA-Seq data (the work is selected for presentation in 1st international Caparica conference in Splicing 2016, Lisbon, Portugal. Manuscript under preparation for the Special Issue on the *The International Journal of Biochemistry & Cell Biology*).

Dominant Negative Isoforms of PPARγ lacking the LBD: mechanism of action

PhD Student 30 th cycle: Anna Sorrentino
Tutor : Dr. Valerio Costa
Affiliation: Institute of Genetics and Biophysics "A. Buzzati-Traverso", CNR, Naples.

PPARγ is a ligand-inducible transcriptional factor master regulator of adipogenesis. It forms a heterodimer with RXRα recognizing responsive sequences (PPRE) in promoter regions of target genes. Loss-of-function mutations in *PPARG* gene are associated with metabolic disorders and cancer. Mutant receptors without functional ligand binding domain show compromised transactivation ability and act as dominant-negative toward wild-type receptor competing for DNA binding and/or RXRα and cofactors' interactions. In recent years, our group identified two PPARγ isoforms lacking the LBD (PPARγΔ_{LBD}) with dominant-negative activity, expressed in normal as well as in cancerous tissues/cells.

The aim of my PhD project is to assess how these PPARγΔ_{LBD} isoforms act as dominant-negative interfering with PPARγ activity. Analysis of RNA-Seq data available in lab revealed that over-expression of PPARγΔ_{LBD} in HEK293 cell line determines a significant alteration of the PPARγ transcriptional program. Preliminary luciferase and Co-IP assays also suggest that these isoforms interact with RXRα. Further analyses to assess PPARγΔ_{LBD} ability to bind DNA and/or to interact with PPARγ partners are on-going. In the last year I also plan to clarify the role of these new PPARγΔ_{LBD} isoforms in PPARγ signalling pathway, possibly evaluating the potential pathological effects of their altered expression.

Reconstructing a Genetic Network from Gene Perturbations in Secretory Pathway of Cancer Cell Lines

PhD Student 30 th cycle: Marina Piccirillo
Tutor : Dr. Mario Rosario Guarracino
Affiliation: Laboratory for Genomics, Transcriptomics and Proteomics (Lab-GTP), High Performance Computing and Networking Institute (ICAR), National Research Council (CNR), Naples, Italy

Gene perturbation studies play an important role in reconstruction of genetic networks and in determining the influence of genes on each other activities. According to this hypothesis, we planned to develop new analysis methods, based on novel algorithms, to reconstruct genetic networks by incorporating gene expression datasets, containing profiles of cell lines that have been exposed to genetic perturbations. In the present work, we focus on a list of genes, localized in secretory pathway which are responsible for the delivery of different kind of proteins from their site of synthesis to their proper cellular location. Using data from high-throughput experiments, gene expression profiles are collected from 33 genes perturbations (knock-down and over-expressed) experiments in 4 cancer cell lines.

Data have been downloaded from the Library of Integrated Network-Based Cellular Signatures (LINCS). We characterized gene regulatory networks (GRNs) of secretory pathway, and we provided some empirical results of the network modular organization. The interesting observation is that all these regulatory genes are also connected with each other through hub nodes. It means that interactions do not have a separate entity and are not regulated by independent behavior of perturbed genes, but probably, there is a global effect of all these perturbations on all sub networks present in an interaction network.

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

PhD Student 29 th cycle: Andrea Oneglia
Tutor : Prof. Andrea Riccio
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The gamete-of-origin-dependent expression of about a hundred imprinted genes is controlled by differential DNA methylation that is established on Imprinting Control Regions (ICRs) during male and female gametogenesis and maintained during replication, despite the genome-wide demethylation process occurring pre-implantation. The protein ZFP57 that binds to the methylated allele of the ICRs by recognizing a [TG]GCmCGC motif has been shown to be required for imprinting maintenance in early mouse embryo and embryonic stem cells (ESCs). We have looked for a possible link between BMP-SMAD signaling and imprinting maintenance in mouse ESCs. By compiling recently published ChIP-seq data, we first observed that SMAD1/5 bind to most ICRs in mouse ESCs. By performing the ChIP in reciprocal hybrid ES strains, we then demonstrated that the binding to the ICRs is parental origin-specific. The analysis of the immunoprecipitated DNA showed that SMAD1/5 bind to the methylated allele of the ICRs. We then performed ChIP-seq for SMAD1/5 and ZFP57 in the same ESC line and demonstrated that these transcription factors share about a hundred target sites including all ICRs. By employing ESC lines in which the Zfp57 gene is inactivated, we further demonstrated that SMAD1/5 require ZFP57 to bind to the common target sites. Experiments are in progress to confirm the ZFP57-SMAD1/5 interaction by co-immunoprecipitation and to investigate the role of SMAD1/5 in imprinting control.

Characterization of a *Mycobacterium smegmatis* TetR-like protein

PhD Student 29 th cycle: Filomena Perrone
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Mycobacterium tuberculosis (Mtb) is one of the most important human pathogens, being spread in about 1/3 of the whole world population. Pathogenic mechanisms, including the ability to survive in macrophages are poorly understood. In a recent study, conducted under acid-nitrosative multi-stress conditions that simulated the phagosomal environment, two genes, *MSMEG_3765* in *M. smegmatis* and its ortholog *Rv1685*c in Mtb, were induced. These genes are annotated as TetR transcriptional regulators and their deduced aa sequences share 74% identity. Members of this family regulate a wide range of cellular activities, including osmotic stress, multidrug resistance, efflux pumps, virulence and pathogenicity. Results of a transcriptional analysis show that *MSMEG_3765* is cotranscribed with *MSMEG_3762/63*, coding for ABC transporters; *MSMEG_3765* codes for a repressor as shown by RTqPCR comparative analysis in the wild type, a *ΔMSMEG_3765* mutant strain, and a complemented strain. TetR-regulated promoter regions upstream of either the *Rv1687c/86c/85c* operon or the *MSMEG_3762/63/65* operon were identified by GFP assays in *M. smegmatis* wt and deletion mutant, showing that the MSMEG3765 protein may bind the *M. tuberculosis* TetR-target sequence. In acid-nitrosative stress conditions the expression of the *MSMEG_3762/63/65* operon is induced, suggesting the involvement of the MSMEG3762/63 efflux pump in stress response.

Study of a CTCF mutant able to bind methylated DNA

PhD Student 29th cycle: Marino Maria Michela
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The CCCTC binding factor (CTCF) is an evolutionarily conserved transcription factor that is involved in various aspects of gene regulation. It was originally identified for its ability to specifically bind regulatory sequences in the promoter-proximal region of the MYC oncogene and in the silencer element of the chicken lysozyme gene. CTCF contains 11 zinc finger (ZF) domains, the first 10 of the classical Cys2-His2 types. The most attractive of all CTCF functions is the ability to mediate insulator function. CTCF-dependent insulators have been particularly characterized in the chicken b-globin locus and in the imprinted Igf2/H19 locus in mouse and human. The minimal DNA binding domain of CTCF is constituted by the region comprising the zinc fingers from 4 to 8 (ZFs 4-8). The methylation of the cytosines present in the CTCF binding sites, as the ones on the paternal allele of the locus Igf2/H19 affects the binding of the CTCF zinc finger 7. In this study, we have produced a CTCF mutant able to bind methylated DNA both in vitro and in Wit 49 cell line in which the locus Igf2/H19 resulted methylated on both maternal and paternal alleles. The CTCF mutant produced has a valine in place of the aspartate in position 451 in the ZF7. Preliminary results of the interactome of the CTCF protein in Wit 49 cells will also be reported,

Session 5. Cancer biology and Immunology

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 is a promising immunotherapeutic strategy and has shown promising results in recent trials.
It is based on redirecting bulk populations of T cells against tumor antigens by introduction of tumor specific TCR. One of the main challenges of this therapy is to limit the off-target toxicity, a consequence of the mispairing phenomenon that can occur when endogenous α and β TCR chains incorrectly pair with the introduced ones. The TCR-mispairing reduces the expression of the therapeutic TCR and may result in auto-immunity.
The aim of my research is to propose a strategy which enhances the correct pairing of the transduced chains.
Mixed dimers formation and functional activity of engineered cells were assessed on mouse models. Obtained results show that the proposed strategy avoids the TCR mispairing and increase T cells functional activity representing a good tool to improve the efficacy of the adoptive TCR gene therapy.

Assessment of Left Ventricular Remodeling by Gated SPECT Myocardial Perfusion Imaging in Diabetic Patients: a Propensity Matched Cohort Analysis

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Aim: Left ventricular (LV) geometry is important in the pathophysiology of heart failure. Diabetes is an independent risk factor for heart failure. Measures of LV remodeling derived from G-SPECT provide incremental and independent information for the identification of patients with heart failure. The aim of this study was to compare LV shape parameters in a propensity score-matched cohort of diabetic and non-diabetic patients with normal myocardial perfusion imaging.
Materials and Methods: We evaluated 1168 patients (806 non-diabetic and 362 diabetic) without history of coronary artery disease (CAD), with normal myocardial perfusion at G-SPECT. To account for differences in baseline characteristics between diabetics and non-diabetics patients, we created a propensity score-matched cohort considering clinical variables. An automated software program was used to calculate parameters indicating shape distortion (stress end-diastolic, SIED, stress end-systolic, SIES, shape index and eccentricity index).
Results: Before matching, diabetic patients were younger with a greater body mass index and higher prevalence of male gender, hypertension, dyslipidemia, smoking, family history of CAD and chest pain (all $P<0.001$). After matching, clinical characteristics were comparable in 332 diabetic and 332 non-diabetic patients. Diabetic patients showed significantly higher SIED and SIES values and lower eccentricity index compared to non-diabetic patients (all $P<0.001$).

Glioblastoma: The role of REST and molecular compounds targeting tumor cells

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Glioblastoma multiforme (GBM or grade IV glioma) is the most common malignant and aggressive type of brain tumor originated from astrocytes. Pathogenetic mechanisms are largely unknown and current treatments are poorly effective.
Rapid migration and aggressive invasiveness are major pathobiological characteristics of GBM that contribute to the malignancy and resistance to therapy.
Few genes have been consistently identified as prognostic biomarkers of GBM. Recently, an oncogenic role of REST in neural tumors has been suggested, with REST up-regulation able to drive cell proliferation and suppress differentiation. Over 30% of human glioblastomas depend on high REST expression for their growth and invasive properties.
The purpose of my PhD project is to investigate the contribution of REST to GBM pathogenesis and to assess the effects of specific molecules, such as HDAC inhibitors, and natural extracts on the proliferation, migration, invasion, angiogenesis and vasculogenic mimicry.
I started an investigation to study how HDAC inhibitors and Ruta extract are able to affect cell motility, invasion and vasculogenic mimicry. Preliminary results suggest that nanomolar concentration of HDAC inhibitors, MS275, MC1568 and TSA reduce the chemotactic response to FBS of glioblastoma cells

Expression and functional characterization of ultraconserved non-coding regions 339+ and 8+ in bladder cancer.

PhD Student 30 th cycle: Sara Terreri
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Bladder cancer (BlCa) is a common malignancy disease. It is still debated whether biomarkers can be used to predict response to different types of therapy, prognosis or recurrence. Studies have shown that lncRNAs can display specific expression patterns in particular cancer, making them a promising tool for diagnosis/prognosis. We recently reported the involvement of a new class of lncRNAs in BlCa, T-UCRs. T-UCR 8+ is overexpressed in BlCa patients, and inversely related to BlCa grade, paving the way for clinical applications. It is localized in the cytoplasm of J82 BlCa cells, suggesting that active molecular exportation could take place and be involved in cancer formation and/or progression. My aim is to clarify the cytoplasmic function of T-UCR 8+ during tumorigenesis and understand its role. I plan to dissect T-UCR 8+ protein network interaction using ChIRP–MS and RAP–MS methods. I also did ISH experiments to reveal the specific localization of another T-UCR overexpressed in BlCa, T-UCR 339+. Preliminary data, showed an abundant presence of it in brain and intestinal tract, and a slight signal in lung, thyroid and bladder. To confirm its abundance I performed RT-PCR in different adult and embryo tissues. Recent efforts to inactivate lncRNAs in mouse models have shed light on their functions indicating important roles. Then I started to generate a knockout mouse model to provide valuable clues about T-UCR 339+ functions.

Analysing tumor-stroma crosstalk through the application of a novel 3D Organotypic invasion assay

PhD Student 30 th cycle: Stefania Belli
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Studying cellular migration and invasion in a 2D context is quite reductive because the locomotory behavior of cells markedly differs in 3D environments. Besides, it was demonstrated that cells of tumor microenvironment, in particular fibroblasts, play a critical role in tumor establishment and progression. My Thesis work is directed to investigate the molecular mechanisms modulating tumor cell invasion in a 3D context, in presence of fibroblasts. In particular, I am focusing on the effects of novel urokinase (uPA)-derived peptides blocking tumor cell migration/invasion as well as on the underlying mechanisms.
In vitro, two peptides (linear and cyclic) inhibit both HT1080 fibrosarcoma cell line and human dermal fibroblasts (TIFs) migration, through their specific binding to α_V integrin subunit.
If TIFs are exposed to one of these peptides or silenced for the expression of α_V integrin, the ability of fibroblasts to organize collagen matrix is impaired, showing that α_V integrin is required. Similary, pre-exposure to one of the two peptides or α_V silencing in TIFs prevents HT1080 invasion in the organotypic culture. Also, TIFs treated with these peptides exhibit a downregulation of α-SMA and an upregulation of caveolin levels, both markers of cancer-associated fibroblasts (CAF) phenotype. In conclusion, these data suggest that fibroblasts play an active role in the modulation of tumoral invasion, and that we can interfere with neoplastic cells-fibroblasts crosstalk.

How different adjuvants and immunization protocols affect the immune response induced by Alzheimer's Disease vaccine (1-11)E2.

PhD Student 29 th cycle: Francesca Mantile

Tutor : Dr. Antonella Prisco
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The primary aim of vaccination is the induction of an appropriate immune response to an antigen and the development of a long-lasting protective immune memory. The design of a new vaccine requires the identification of the best antigen-adjuvant combination, as well as the optimal timing and number of recall doses required to maintain the antibody titer above the protective threshold. In this study we have analyzed how different adjuvants and immunization protocols can affect the immune response induced by multimeric protein vaccine (1-11)E2, a vaccine for Alzheimer's Disease designed to induce antibodies agaist the beta-amyloid. We compared the kinetic and the quality of the antibody response elicited by vaccine (1-11)E2 when formulated with different adjuvants. Then we have studied the effect of different vaccination schedules on the primary immune response and immunological memory. We show that adjuvants alter the magnitude and avidity of the antibody response without changing the antibody isotype distribution. We show that the immune response elicited by vaccine (1-11)E2 undergoes a consolidation phase. During this phase a second dose of vaccine can disrupt the development of immune memory.

Differential intestinal cell phenotype and cytokine profiles in overt and potential celiac disease

PhD Student 29 th cycle: Serena Vitale
Tutor : Dr. Carmen Gianfrani

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Celiac disease (CD) is an immune-mediated disorder induced by gluten. Two main disease forms are known: full-blown CD, with positive anti-tissue transglutaminase antibodies and small intestinal villous atrophy, and potential CD with positive serology but normal mucosa. The cytokine profile and the phenotype of intestinal T-cells from children affected by these two forms of CD were investigated.
Jejunal biopsies were obtained from 19 children with acute CD, 16 with potential CD and 12 non-CD controls. Phenotype and cytokine production patterns were analysed by flow cytometry, in gluten-raised T-cell lines (TCLs) and in freshly mucosal cells.
A significant increased number of CD4CD8 double negative CD3 TCRγδ+ cells was found in TCLs from acute CD compared to potential CD or non-CD healthy subjects. A higher fraction of IL-4 producing cells was detected in TCLs from children with normal mucosa (either potential CD or controls). These data were confirmed by the ex-vivo analysis on freshly isolated intestinal cells.
In conclusion, we found that in young CD patients the villous atrophy correlates with a marked expansion of TCRγδ+ T-cells and the disappearance of IL-4 producing cells.
The IL-4 and TCRγδ+ T-cells could represent new biomarkers to support the serological and histological diagnosis of CD.