

Anno accademico 2011/2012

Dottorato in Biochimica e Biologia Molecolare

Elenco delle presentazioni dei dottorandi per l' idoneità a sostenere la tesi finale di dottorato

- Giovedì 25 Ottobre ore 9.30, Aula D29: 20 min+10 min discussione

III anno XXV ciclo:

Cozzoli Eliana
Italiano Dafne
Mastrangelo Nicolina
Lenti Elisa

Memmi Elisa Maria (presenta il 24 ottobre)

Relazioni attività di ricerca - III° Anno

Eliana Cozzoli, XXV ciclo
Tutor: Prof. Nicola Rosato
Co-tutor: Prof.ssa Alessandra Gambacurta

BDSCs plasticity: towards the land of Tir Nan Og

BDSCs (Blood Derived Stem Cells) obtained from peripheral blood represent a non-controversial and promising source of adult stem cells that are readily available because their plasticity to differentiate in several cell types. We used a simple and fast deprogramming method that allows us to obtain, from few millilitres of peripheral blood, a population of pluripotent stem cells after sorting. To confirm this deprogramming process we tested the presence of the main pluripotency markers Oct4, Sox2 and Nanog. Moreover, we have demonstrated that deprogrammed cells are able to be reprogrammed and to differentiate in several cell lines representative of the three embryonic sheets like osteocytes (mesoderm), neurons (ectoderm) and hepatocytes (endoderm). Our challenge has been to explain the deprogramming and reprogramming pathways starting from Nanog, the eternal youth molecule, and going through the others key molecules involved in these processes. Through the celtic legend of Tir Nan Og we tackle the events that drive a cell to go back and forth, although backwards and forwards could be swapped, it depends on the point of view.

Dafne Italiano, XXV Ciclo
Docente guida: Prof. Gerry Melino

Identification of NCF2 as a novel target of p53

Analysis of two microarrays performed in inducible SAOS clones for p53, TAp63 α and Δ Np63 α expression allowed to notice the increase in the expression of NCF2, cytosolic subunit of the NADPH Oxidase enzyme complex, in response to the induction of p53. The recruiting of NCF2 to the cell membrane causes the activation of the complex and thus the generation of superoxide from molecular oxygen. NCF2 promoter activity assays were performed, confirming the activation by p53 and, to a lower extent, by TAp63 α . Three putative binding sites for p53 on the promoter were predicted using a bioinformatic tool, and the subsequent chromatin immunoprecipitation (ChIP) assay showed the binding of the

transcription factor to at least one of the sites, thus confirming the hypothesis. The regulation was also confirmed by Real Time PCR in several cell lines and after the activation of p53 by DNA damage, an effect that is surely dependent on p53 since it is not found in absence of the transcription factor. It was then examined the effect of NCF2 overexpression and silencing on ROS levels and apoptosis in different cell lines, showing that while the overexpression has little effect, silencing causes a great increase in apoptosis levels in both p53 negative and positive cells. It is then interesting to evaluate the levels of cellular stress that lead to changes in the expression of NCF2, to fit these results in the picture of the anti- or pro-oxidant functions of p53.

Nicolina Mastrangelo, XXV ciclo
Docente guida: Prof. Alessandro Finazzi-Agrò
Tutor: Dott.ssa Monica Bari

Alterations of the endocannabinoid system in Huntington's disease: in vitro and in vivo studies

Endocannabinoids, like AEA and 2-AG, their biosynthetic (NAPE-PLD and DAGL, respectively) and hydrolytic (FAAH and MAGL, respectively) enzymes, and their receptors (CB1, CB2, TRPV1) form the so-called Endocannabinoid System (ECS), note to be involved in Huntington Disease (HD). In my work I analyzed the expression and the activity of ECS proteins, as well as the eCBs levels in whole brain and in different brain areas, striatum and cortex of R6/2 mice, a widely used model of HD and in rat HD43 cells, an inducible cellular model of HD. HD43 cells (deriving from striatal ST14A cells) express the N-terminal 548aa portion of mutant *htt* gene upon induction by doxycycline. Also, I observed the FAAH activity in lymphocytes of R6/2 mice, in order to evaluate whether central ECS alterations were mirrored by peripheral cells.

The results showed a reduction of NAPE-PLD and DAGL activity, and of CB binding, as well as an increase in 2-AG content, without any other change in ECS elements, in whole brain of 12-week-old R6/2 mice confronting to their wild-type littermates; the presence of a fully functional ECS in HD43 cells upon induced and non-induced conditions, and decrease in FAAH activity, already observed in human brain and lymphocytes of HD patients. Interestingly, striatum seems to be the area mainly responsible of the ECS observed changes. On the whole, this data suggest that ECS is differently affected in mouse and human HD, and that HD43 cells are suitable for high throughput screening of FAAH-oriented drugs affecting HD progression.

Abbreviations: **AEA**, *N*-arachidonylethanolamine; **2-AG**, 2-arachidonoylglycerol; **CB1/2**, type-1/2 cannabinoid receptor; **DAGL**, diacylglycerol lipase; **eCBs**, endocannabinoids; **ECS**, endocannabinoid system; **FAAH**, fatty acid amide hydrolase; **HD**, Huntington's disease; **htt**, huntingtin; **MAGL**, monoacylglycerol lipase; **NAPE-PLD**, *N*-acyl-phosphatidylethanolamines-hydrolyzing phospholipase D; **TRPV1**, transient receptor potential vanilloid-1.

Elisa Lenti, XXV ciclo
Docente guida: Dott.ssa Francesca Bernassola

The oncogenic transcription factor Tlx1 controls spleen development through regulation of the retinoic acid pathway

The oncogenic transcription factor Tlx1/Hox11 promotes cellular proliferation during organogenesis and its loss of function mutation in mice causes spleen agenesis. Although few Tlx1 target genes have been identified, the downstream transcriptional networks controlled by Tlx1 during organ development remain mostly unknown. To this end, we performed DNA-microarray coupled to ChIP-Seq analysis and found that loss of Tlx1 causes deregulation of several pathways associated with organ growth and cancer, including the Retinoic Acid pathway. By using *in vivo* and *in vitro* approaches we confirmed that Tlx1 balances RA levels by controlling the expression of genes involved in RA synthesis, transport and nuclear receptors. In addition, we found that Cyp26b1, an enzyme involved in RA degradation was severely down-regulated in the absence of Tlx1 and that loss of it in mice causes asplenia. These findings indicate that Tlx1 finely tunes RA levels to assure organ expansion. We foresee that identification of physiological Tlx1 controlled pathways will shed light on the mechanisms by which ectopic activation of this oncogene in T-ALL induces thymocyte maturation arrest and pre-leukemia onset.

Elenco delle presentazioni dei dottorandi per il passaggio di annualita'

- Mercoledì 24 Ottobre ore 9.30 Aula D29: 10 min+5 min

III anno XXV ciclo:

Memmi Elisa Maria

II anno, XXVI ciclo:

Arno' Barbara
Evangelista Daniela
Latina Alessia
Mancini Mara
Loria Rossella
Iorio Egidio
Chimenti Maria Sole
Pinetti Valentina
Shantaram Katkar Prafulla
Talamonti Emanuela

Schillaci Carlo, passa a studente part-time, posticipa al prossimo anno presentazione ed esame

El Said Dalya, assente giustificata

I anno, XXVII ciclo:

Farrotti Andrea
Giacobbe Arianna
Palombo Ramona
Cefalù Sebastiano
Vieira Sara
Adanti Sara
Bisogno Tiziana
Carfora Virginia

Cafolla Clodomiro, chiesta sospensione per altro corso universitario (da sett '12)
Bianculli Antonio Giuseppe, chiesta sospensione per altro corso universitario (da sett '12)

Relazioni attività di ricerca - III anno XXV ciclo:

Elisa Maria Memmi, XXV ciclo
Docente guida: Dott.ssa Francesca Bernassola

Role of p63 in regulating the self-renewal properties of mammary epithelial cancer stem cells

p63, a structural and functional homologue of the p53 transcription factor, is required for maintaining the proliferative potential of both normal and cancer epithelial stem cells. Up to now, whether or not p63 plays a role in controlling the mammary cancer stem cell (cSC) phenotype is still poorly elucidated. Our study aims to investigate the involvement of p63 in regulating the self-replicative properties of mammary cSCs, and to identify the molecular mechanisms underlying p63 action. To this aim, we have exploited the MMTV-ERBb2 transgenic mouse as a stem cell model of mammary carcinogenesis. The analysis of ERBb2 tumors revealed that the positivity for Sca-1, a cSCs marker, is associated with p63 expression in a subset of cSCs. In addition, p63 is up-regulated in mammosphere cultures of breast cSCs, as compared to the normal mammary gland. Remarkably, purified cSCs display higher expression levels of p63 than the progenitor population. Lentiviral-mediated delivery of shRNA against p63 in tumor mammospheres significantly reduces their size as well as inhibit their self-renewal ability. Importantly, p53 is dispensable for p63 to promote self-renewal of cSCs, since p63 down-regulation in p53 knock-out mammospheres decreases the cSC proliferative at the same extent as in the ERBb2 mammospheres. Our findings demonstrate that p63 identifies a mammary cSC population, which possesses increased replicative potential, and that p63 likely acts through its own transcriptional program, rather via the inhibition of p53.

Relazioni attività di ricerca - II° Anno

Barbara Arnò, XXVI ciclo

Docente guida: Prof. Alessandro Desideri

Investigation of a human-Plasmodium Topoisomerase IB chimera

DNA topoisomerases catalyze changes in DNA topology via the formation of a transient covalent enzyme-DNA intermediate. Human Topoisomerase IB (Top1) is made by four domains: N-terminal, core, linker and C-terminal domain. The enzyme is of significant chemical interest since it's the only target of anti cancer drug such as camptothecin (CPT). The same protein in the *Plasmodium falciparum* is a 839 aminoacids monomer encoded by a single copy gene that shows a 42% identity with the human homologue. *Plasmodium falciparum* topoisomerase IB (PfTop1) contains three insertion regions when compared to Top1: two in the core and one in the linker domain. To address the functional consequences of the different lenght of the linker domain of the PfTop1, a human/malaria chimera, swapping the Top1 linker domain with the corresponding one of PfTop1, has been produced. The chimera displays a much faster religation rate when compared to Top1. The chimera is also CPT resistant, a property correlated to its fast religation rate, likely due to a large linker flexibility as confirmed by MD simulations. These data are in line with previous investigations. Interestingly overexpression of the chimera in yeast is toxic suggesting that the presence of a long linker induces a different recognition among various molecular partners that bring the yeast to die. This phenomenon will be further investigated and the effect of the chimera on human cells will be studied as well.

Evangelista Daniela, XXVI ciclo

Docente guida: Prof.ssa Luciana Avigliano

2-Arachidonoylglycerol (2-AG) modulates endothelial/leukocyte interactions by controlling selectin expression

The immuno-modulatory role of endocannabinoids (ECs) is still under debate; moreover literature data mainly relate to single cell types or analysis of late stages of the inflammatory process. We characterized the effect of ECs in early events of endothelium (ECV304)/leukocyte (Jurkat) interactions. RT-PCR, Western blot, ELISA and confocal microscopy analysis showed that 2-AG was able to initiate and complete the leukocyte adhesion cascade. A short exposure of ECV304 to 2-AG primed them towards a pro-inflammatory state, through exposure of specific selectins. Commitment to inflammation was permanent, since activated ECV304 released tumour necrosis factor- α (TNF- α) for up to 24 hours, despite the removal of 2-AG after 1 hour of incubation. TNF- α -containing medium, derived from 2-AG-treated ECV304, promoted leukocyte recruitment: conditioned media and co-culture studies showed that Jurkat cells enhanced the expression of L-selectin and P-selectin-glycoprotein-ligand-1, and increased adhesion and trans-migration. 2-AG indirectly promotes leukocyte recruitment into inflamed sites by acting on endothelial cells, thus representing a potential therapeutic target for treatment of inflammatory diseases.

Alessia Latina, XXVI ciclo
Docente guida: Prof. Gerry Melino

Identification of new targets of p63 in different metabolic pathways

The transcription factor $\Delta Np63\alpha$ has an important role in epidermal development (proliferation and differentiation). Its expression level varies in the different layers of the epidermis, as it is abundant in the basal layer and decreases in the upper layers. To identify new targets of p63, we used the RT² Profiler PCR Array. This approach takes advantage of the combination of Real Time PCR performance and the ability of microarrays to detect simultaneously the expression of many genes. In every plate there are 84 genes related to a pathological condition or a biological pathway. We silenced TP63 in keratinocytes and we analyzed the expression of the genes belonging to specific pathways. The plate that presented the most differences compared to the control cells was the oxidative stress and antioxidant defense one. In particular, after silencing of TP63, 13 genes were downregulated and 8 were upregulated. We focused our attention on one CYGB, which codes for cytoglobin, a member of the globin protein family that facilitates diffusion of oxygen through tissues and acts as a scavenger for nitric oxide or other reactive oxygen species. Further investigations will be necessary to confirm the direct regulation of CYGB by $\Delta Np63\alpha$ (by promoter activity assay, real-time PCR and western blot) and to evaluate the biological role of this regulatory mechanism in keratinocytes.

Mara Mancini, XXVI ciclo
Docente guida: Dott.ssa Eleonora Candi

MicroRNAs participate to human dermal fibroblasts senescence acting on cell adhesion and remodeling of the extracellular matrix

Ageing of human skin is associated with phenotypic changes in the cutaneous cells; the major functional markers of ageing occur as consequences of dermal and epidermal cell senescence and of structural and compositional remodeling of normally long-lived dermal extracellular

matrix proteins. Understanding the contribution of the dermal cells in skin ageing is a key question, since this tissue is particularly important for skin integrity and its properties can affect the epidermis. Several microRNAs have been shown to be involved in the regulation of pathways involved in cellular senescence and exerted important effects on tissues ageing. In this study, we demonstrate that the expression of miR-152 and miR-181a increased during the human dermal fibroblasts senescence and that their overexpression, is sufficient to induce cellular senescence in early-passage cells. The increase of these miRNAs during cells senescence was accompanied by a decrease in integrin $\alpha 5$ and collagen XVI expression at mRNA and/or protein levels resulting in reduced cellular adhesion and suggesting extracellular matrix remodeling. These findings indicate that changes in miRNAs expression, by modulating the levels of adhesion proteins and extra-cellular matrix components, such as integrin $\alpha 5$ and collagen XVI, could contribute to the compositional remodelling of the dermis and epidermis occurring during skin aging.

Rossella Loria, XXVI ciclo

Tutor: Dott.ssa Rita Falcioni

Docente guida: Dott.ssa Eleonora Candi

Role of SEMA6A/Plexin/MICAL-1 complex in melanoma BRAF metastatization

Melanoma is a cancer that arises from melanocytes, specialized pigmented cells that are found predominantly in the skin. The Ras/Raf/MEK/ERK pathway is a key regulator of melanoma cell proliferation. This pathway is activated through mutations in NRAS (Q61L), or in BRAF (V600E). BRAF mutations in melanoma are more prevalent than NRAS. We have compared the gene profile of BRAF and NRAS-mutated human melanoma cell lines by microarray analysis. Interestingly, our data show for the first time a strong up regulation of the semaphorin 6A (SEMA6A), only in BRAF melanoma. The SEMA6A signal is mediated by plexins, a family of cell surface receptors that plays an important role modulating cytoskeletal organization and as consequence motility and invasion. This is achieved, in part by the interaction of SEMA6A/Plexin complex with molecule-interacting CasL (MICAL) that was also up-regulated in BRAF melanoma and which function is strongly related to remodeling of cytoskeleton and apoptosis.

On the basis of the above reported results I propose: to investigate in detail the role of SEMA6A in cytoskeletal remodelling, thus consequently affecting cell shape, cell motility, and invasion in BRAF melanoma; to investigate the role of MICAL-1 that, in concert with the complex SEMA6A/Plexin, inhibits the apoptosis by the inhibition of phosphorylation of NDR kinase; to analyze SEMA6A in specimens of NRAS and BRAF melanoma derived from patients surgically treated in Istituto Tumori di Milano and Istituto Regina Elena for Cancer Research in Rome.

Egidio Iorio, XXVI ciclo

Docente guida: Prof. Maurizio Paci

Effects of downmodulation of choline Kinase and phosphatidylcholine-specific phospholipase C on the Magnetic Resonance Spectroscopy choline profile of epithelial ovarian and breast cancers

Magnetic resonance spectroscopy (MRS) allows detection of abnormal profiles of aqueous choline-containing metabolites (tCho) of the phosphatidylcholine (PtdCho) cycle in cancer cells and tissues at preclinical and clinical level. Our previous studies showed a significant increase in phosphocholine (PCho) in epithelial ovarian cancer (EOC) cells compared with

non tumoral counterparts (Iorio E et al, Cancer Res 2005), associated with 12-to-25-fold activation of choline kinase (ChoK) and 5-to-17-fold activation of PtdCho-specific phospholipase C (plc). In view of the predominant role of the ChoK α isoform in steering the overall ChoK activity, we specifically silenced the CHKA gene expression by both transient and stable RNA interference in two EOC cell lines (SKOV3 and INTOV11) and evaluated the main biological effects on cell proliferation and alterations of the tCho profile (Granata et al, 2012 submitted). Pharmacological inhibition of PtdCho-plc induced a 30-to-40% reduction of PCho content and blocked cell proliferation in two EOC cell lines. Our observations, confirmed the major role of ChoK and PtdCho-plc enzymes in the deregulated choline metabolism of EOC cells and suggested these enzymes as promising targets for anticancer treatment. These studies are currently being extended to breast cancer cells to investigate possible mechanisms of cross-talk between these enzymes.

Maria Sole Chimenti, XXVI ciclo
Docente guida: Prof. Gerry Melino

Multiplatform metabolomic study of CD4+ T-Lymphocytes after Methotrexate and Infliximab treatment: focus on rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by infiltration of polymorphonuclear cells and TCD4+ in the joints with the development of synovial inflammation that results in destruction of articular cartilage and adjacent bone. TCD4+ cells comprise a large proportion of the cells that invade the synovium and may therefore be a cell type of pathogenic importance. Inflammatory mediators are implicated in the establishment and progression of inflammatory joint destruction, including proinflammatory cytokines as TNF α , IL-6, and IL-17. Serum concentrations of IL-17, IL-23, IL-6 e TNF α were tested in 15 RA patients and in 15 PsA patients before and after 22 and 54 weeks of TNF α inhibitors treatment. Data were correlated to levels of VitamineD, Osteoprotegerin, beta-crosslaps and with clinical assessment. RA treatments include DMARDs, as methotrexate (MTX), that is characterized by several side effects and TNF α inhibitors that have demonstrated significant efficacy and safety profile. The purpose of our study was to evaluate in TCD4+, from a multiplatform metabolomic study, treatment with MTX and infliximab in order to investigate the metabolomic features in healthy subjects and in RA patients. Results will be correlated with clinical and inflammatory findings in order to associate metabolomic results to drugs efficacy and tolerability.

Valentina Pinetti, XXVI ciclo
Tutor: Prof. Antonio Costanzo
Docente guida: Dott. Alessandro Terrinoni

Developmental factor IRF6 exhibits tumor suppressor activity in squamous cell carcinomas

The transcription factor IRF6 regulates craniofacial development and is a component of a regulatory feedback loop that controls the proliferation of epidermal cells, being transcriptionally activated by p63 and inducing its proteasome-mediated downregulation. We supposed that IRF6 may also be involved in skin carcinogenesis so we analyzed IRF6 expression in squamous cell carcinomas (SCCs) finding downregulation of IRF6 that

correlates with methylation on a CpG island located in its promoter region. By combining ChIP-seq for IRF6 binding sites and gene expression profiling in primary human keratinocytes after IRF6 depletion by siRNAs, we found that IRF6 bound genomic regions are enriched in adhesion and proliferation genes. We also showed *in vitro* that IRF6 downregulation promotes invasive behavior and its reintroduction into SCCs inhibits cell growth. Our results indicate a function for IRF6 as suppressor gene in stratified epithelia. Moreover, IRF6 binds regions near miRNAs which are involved in regulating the pluripotency reprogramming of stem cells. Some of these are also differentially expressed in cancer stem cells. Therefore, our future plan is to study the role of IRF6 as regulator of stemness and pluripotency.

Prafulla Katkar, XXVI ciclo

Docente guida: Prof. Alessandro Desideri

A Natural Anticancer Agent Thaspine Targets Human Topoisomerase IB

The different steps of the topoisomerase I catalytic cycle have been analyzed in the presence of the plant alkaloid thaspine (1-(2-(Dimethylamino)ethyl)-3,8-dimethoxychromeno[5,4,3-cde]chromene-5,10-dione), known to induce apoptosis in colon carcinoma cells. The experiments indicate that thaspine inhibits both the cleavage and the religation steps of the enzyme reaction. The inhibition is reversible and the effect is enhanced upon pre-incubation. Molecular docking simulations of thaspine over topoisomerase I, in the presence or absence of the DNA substrate, show that thaspine, when interacting with the enzyme alone in the closed or in the open state, can bind in proximity of the active residues preventing the cleavage reaction, whilst when docked with the enzyme-DNA cleavable complex intercalates between the DNA bases in a way similar to that found for camptothecin, explaining its religation inhibition. These results unequivocally demonstrate that thaspine targets human topoisomerase I.

Emanuela Talamonti, XXVI ciclo

Docente guida: Prof.ssa Filomena Fezza

Novel modulators of inflammasomes: insights from the endocannabinoid system

Innate immunity is characterized by its ability to recognize a wide range of pathogens through a limited number of receptors, mainly TLRs, and the recent NLRs, which consist of soluble proteins that survey the cytoplasm for "danger signals" that advertise the presence of intracellular invaders, forming the inflammasomes. Inflammasomes are molecular complexes that activate inflammatory caspases, which are involved in the maturation of cytokines of the IL-1 family. On the basis of the recent evidences demonstrating the important role of the endocannabinoid system in the modulation of several immune responses, we investigated whether endocannabinoids may interfere with inflammasomes activation. In particular, during the second year of PhD, we found that endocannabinoid anandamide (AEA) inhibits NLP3-inflammasome-dependent caspases-1 expression and production of IL-1 β , and IL-18 from human macrophages, whereas the other endocannabinoid 2-arachidonoylglycerol (2-AG) acts as a danger signal by inducing NLP3-inflammasome-mediated responses. Overall, our findings account for a new homeostatic role of the endocannabinoid system in the fine-tuning of those feedback loops, which are crucial for either initiation or resolution phases of inflammation, thus hinting at novel therapeutic opportunities for the treatment of several inflammatory diseases.

Relazioni attività di ricerca - I° Anno

Farrotti Andrea, XXVII ciclo
Tutor: Prof. Lorenzo Stella
Docente guida: Prof. Nicola Rosato

*Molecular dynamics simulations applied to biochemical systems.
Effects of pathogenic mutations in proteins of the Ras-MAPK pathway and
mechanism of action of antimicrobial peptides.*

Molecular dynamics (MD) simulations represent a useful tool to reveal atomic details in many different biochemical processes. Combining all-atoms and coarse-grained MD simulations, we investigated two different problems. The first concerns the conformational and dynamics effect of point mutations in proteins of the Ras-MAPK pathway, as R-Ras or SHP2, expressed in Noonan syndrome patients. Our simulations on the V55M R-Ras mutant revealed an increased mobility of the switch I region with respect to the wild-type protein, explaining the experimentally observed nucleotide dissociation. The second topic concerns the mechanism of action of antimicrobial peptides (AMPs), a class of molecules able to kill bacteria mainly by making their membrane permeable. We are using MD simulations to understand their mechanism of pore formation, by analyzing peptide orientation in the membrane. Currently, we are simulating two different AMPs called trichogin GAIV (GAIV) and LAH4. Simulations of GAIV show that this short peptide is able to form transmembrane pores by causing a thinning of the bilayer; preliminary structures obtained for LAH4 show a pH-dependent orientational transition, in agreement with NMR data.

Arianna Giacobbe, XXVII ciclo
Tutor: Dr. Angelo Peschiaroli
Docente guida: Prof. Gerry Melino

*Characterization of Metastasis Suppressor 1, MTSS1, as a novel p63
transcriptional target gene*

Metastasis represents the end product of a multistep cell-biological process in which cancer cells acquire the ability to invade organs anatomically distant from the primary site. Metastasis cascade is modulated by many factors, including metastasis activators and suppressors. Metastasis Suppressor 1 (MTSS1) was originally identified as a metastasis suppressor protein missing in metastatic bladder carcinoma and prostate cancer. MTSS1 is described as an actin polymerization factor and it may be an important player in tumor metastasis. By microarray analysis, we found MTSS1 as a potential target gene of p63, a transcription factor belonging to the p53 family. We found that in normal and in cancer cell lines the ectopic expression of TAp63 isoforms, but not DNp63, induces the expression of MTSS1 both at mRNA and protein levels. By ChIP-seq analysis we found three putative p63 regulatory elements in the MTSS1 locus. We validated two of these regulatory elements by ChIP analysis, luciferase assay and mutagenesis analysis. Next studies will be addressed to investigate the biological relevance of the TAp63-dependent transcription of MTSS1 during cell migration, cell invasion and metastasis.

Ramona Palombo, XXVII ciclo

Docente guida: Prof. Alessandro Terrinoni

Molecular mechanisms underlying skin development and senescence

Replicative senescence in human keratinocyte has an important role in the epidermal tissue together with the differentiation and the proliferating processes, characterized by morphological changes and specific gene expression profiles.

p63, in particular, a homologue of the tumor suppressor p53, is expressed in the basal layers of stratified epithelial tissues. It is responsible for the maintaining of the proliferative potential of keratinocytes, and loss of its expression contributes to induction of senescence in primary human keratinocytes.

To best understanding transcriptional changes that occur in aged keratinocyte rather than proliferating, different strategies can be established. We plan to use both human skin disease and in vitro models. On this side, we are carrying out a whole transcriptome analysis with SOLiD™ system, using proliferating, senescent and siRNA p63 treated keratinocytes.

The Next-Gen sequencing platform will be able to generate a vast amount of sequence data that was previously unobtainable helping us to identify molecular pathways involved in epidermal tissue homeostasis and detect new important targets for human skin diseases.

Sebastiano Cefalù, XXVII ciclo

Docente guida: Dr. Eleonora Candi

Investigation into the possible roles of TAp63 γ isoform in muscle differentiation

p63 is a transcription factor, member of the p53 family. In TP63 gene there are 2 promoters that allow the expression of two principal proteins, including a full length and an amino-deleted isoform, Tap63 and Δ Np63 respectively. Moreover, alternative splicing at the 3'-end produce addition proteins for both the isoforms (alpha, beta and gamma isoforms). Which isoform is responsible for each specific phenotype as stemness, differentiation, cycle arrest, mobility and invasion and senescence still remains unclear. In our work, we have characterized both wild type and TAp63KO mice for tissue-specific expression of all p63 isoforms, by semiquantitative PCR. The most interesting data is the high expression of the isoform TAp63 γ in the skeletal muscle suggesting an its hypothetical role in this specific tissue. In vitro studies, using C2C7 myoblast cell line, which faithfully mimics skeletal muscle differentiation, indicate a clear increase for TAp63 γ during the skeletal muscle differentiation evaluated by quantitative RT-PCR, western blot and immunofluorescence assay. Our goal is to identify TAp63 muscle-specific target genes in order to understand its functional role both in vivo and in vivo.

Sara Vieira, XXVII ciclo

Docente guida: Prof. Alessandro Desideri

Interaction of human DNA topoisomerase IB with new compounds

Human DNA topoisomerases play a key role in cellular processes affecting the topology and organization of DNA such as replication, transcription, recombination and repair. It solves problems related with supercoiled DNA by breaking and rejoining DNA strands. DNA topoisomerases are classified into type I and type II and type I is the only target of camptothecin (CPT), a natural plant alkaloid. Its derivatives are used in cancer

therapy. Anticancer agents can affect several enzymatic mechanisms such as enzyme binding inhibition, DNA cleavage inhibition and stabilization of the covalent complex DNA-topoisomerase. LOM547 is a new compound that showed human DNA topoisomerase IB (hTOPOIB) inhibition. In this year I analyzed the mechanism of LOM547 on relaxation activity and on each step of the catalytic cycle of hTOPOIB. The results demonstrated that LOM547 compound inhibits relaxation of supercoiled and cleavage of DNA substrate, although it does not affect religation reaction. Pre-incubation of hTOPOIB with the compound before adding DNA substrate increases the inhibitor effect. Binding reaction showed that LOM547 does not perturb DNA-enzyme binding.

Sara Adanti, XXVII ciclo

Tutor: Dott.ssa Maria Lucibello – CNR, Istituto di Farmacologia Traslazionale

Docente guida: Prof.ssa Alessandra Gambacurta

Molecular Mechanisms of Carcinogenesis: identification of new targets for diagnosis and therapy

Several mechanisms of stress adaptation in cancer cells provide both a growth/survival advantage and a drug-resistant phenotype. Recently, we have indentified the Translationally Controlled Tumor Protein (TCTP) as a stress-induced survival factor in cancer cells. We have investigated how high levels of TCTP can mediate cell survival pathways, while its reduction **increases sensitivity** to cell-death induced by oxidative and metabolic stress. Our findings show that the aberrant production of TCTP by cancer cells is a crucial event leading to the selection of a stress phenotype by conferring a growth/survival advantage. Indeed, our preliminary *in vitro* data show that the inhibition of TCTP expression levels, in the metastatic breast cancer cell lines MDA-MB-231, increases the sensitivity to chemotherapy. The results obtained show that the cells clones that display higher levels of TCTP are more resistance to treatment with chemotherapy, such as cisplatin, compared to the same cells silenced for TCTP. Our aim is to assess the tumor cell growth to conventional anticancer drugs in TCTP-silenced cancer breast cell lines. The decrease in TCTP activity may redirect tumors cells towards apoptosis and therefore it may represent a target for molecular-based therapies. This possibility could improve conventional chemotherapy treatments.

Clodomiro Cafolla, XXVII ciclo

Docente guida: Dott.ssa Filomena Fezza

Urtica dioica: a study of its anti-proliferative effects

Urtica dioica (UD) is a herbaceous perennial flowering plant, well-known as stinging nettle. The plant has many stinging hairs, trichomes, on its leaves and stems, which, in case of contact, release histamine and other chemical substances such as acetylcholine, serotonin and leukotrienes. It has been used as a medicine and a food source for centuries.

Nowadays, UD extracts are recommended to treat several diseases, such as benign prostatic hyperplasia, diabetes, hemorrhages and osteoarthritis. Only few data are available on its role in preventing and treating cancer.

The aim of the present project is to study whether UD extracts can exert anti-proliferative actions on neoplastic cells.

Firstly, our work has focused on streamlining the procedure of herbal extraction. The different extracts were analyzed by Folin-Ciocalteu Assay and by High Pressure Liquid Chromatography. Secondly, we investigated the effects of UD extracts on cell proliferation and cell death in human prostate cell lines, PC-3 (androgen-unresponsive) and LnCap

(androgen-sensitive). The aqueous extract clearly leads to dose-dependent growth arrest and death of Pc-3 showing a hormone receptor independent pathway.

Next steps will consist in analyzing UD organic extracts and assessing their effects on cell models. Furthermore, we will characterize cell death pathway induced by aqueous extract. Finally, we will test the extracts on other cell lines.

Tiziana Bisogno, XXVII ciclo

Docente guida: Prof. Alessandro Finazzi Agrò

Inhibitors of the metabolism of the endocannabinoid 2-AG: potential therapeutic effects

The development of inhibitors of the biosynthesis of the endocannabinoid (EC) 2-AG via diacylglycerol lipases (DAGL) α and β is just starting to be considered as a novel and promising source of pharmaceuticals for the treatment of disorders that might benefit from a reduction of EC tone, such as hyperphagia in obese subjects. Here it is described the synthesis and pharmacological characterization *in vitro* of four new compounds: 1-((fluoro(methyl)phosphoryl)oxy)-3-(pentyloxy)propan-2-yl-oleate, (O-7458); 1-ethoxy-3-((fluoro(methyl)phosphoryl)oxy)propan-2-yl-oleate, (O-7459); 1-((fluoro(methyl)phosphoryl)oxy)-3-isopropoxypropan-2-yl-oleate, (O-7460); and (S)-1-methoxy-5-oxopentan-2-yl oleate, (O-7344). Of the four compounds, only O-7460, exhibited both high potency against the human DAGL α , and selectivity towards both COS cell monoacylglycerol lipase (MAGL) and rat fatty acid amide hydrolase (FAAH). O-7460 did not exhibit measurable affinity for human recombinant CB₁ or CB₂ cannabinoid receptors. In intact N18TG2 cells stimulated with ionomycin, O-7460 reduced de novo biosynthesized 2-AG levels. In conclusion, O-7460 might be considered a useful pharmacological tool to investigate further the role played by 2-AG both *in vitro* and *in vivo*.

Carfora Virginia, XVII ciclo

Tutor: Dott.ssa Simonetta Amatiste- Istituto zooprofilattico Roma

Docente Guida: Prof.ssa Luciana Avigliano; Prof. Antonello Rossi

Caratterizzazione genotipica di Prototheca spp isolata da campioni di latte bovino

Prototheca è una microalga unicellulare eterotrofa filogeneticamente correlata al genere Chlorella, diffusa negli allevamenti di bovine da latte in particolare in presenza di cattiva igiene ambientale associata ad elevati tassi di umidità. La mastite bovina sostenuta dalla microalga Prototheca è stata segnalata principalmente come patologia mammaria di origine ambientale ad insorgenza sporadica. Lo studio è stato rivolto all'identificazione fenotipica e genotipica del genere Prototheca isolata da campioni di latte bovino.

Per l'identificazione di specie del genere Prototheca è stata sviluppata una Multiplex PCR utilizzando due coppie di primers. La prima coppia è stata utilizzata per il rilevamento dell'rDNA 18S delle seguenti specie di Prototheca: *P. zopfii*, *P. blaschkeae*, *P. moriformis*, *P. ulmea* e *P. stagnora*. La seconda coppia di primers è stata utilizzata per il rilevamento di *P. wickerhamii*. Gli amplificati ottenuti con la prima coppia di primers sono stati sottoposti a sequenziamento. I ceppi di *P. zopfii* identificati tramite sequenziamento sono stati sottoposti a RFLP per la determinazione dei genotipi maggiormente correlati a mastite.

E' stato inoltre previsto di effettuare nel corso del 2° e 3° anno di dottorato uno studio in collaborazione con l'Istituto zooprofilattico sperimentale del Lazio e della Toscana sugli effetti immunomodulanti di sostanze fitochimiche nelle mastopatie infettive del bovino da

latte, analizzando l'attività di fattori di trascrizione (come NF- κ B), nella regolazione della risposta immunitaria.