

Anno accademico 2012/2013

Dottorato in Biochimica e Biologia Molecolare

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

- Lunedì' 28 Ottobre ore 10.30, Aula D29: 20 min+5 min discussione

III anno XXVI ciclo:

Barbara Arno'
Alessia Latina
Maria Sole Chimenti
Hmada Dalya
Iorio Egidio
Daniela Evangelista
Valentina Pinetti
Emanuela Talamonti
Tiziana Bisogno

Assente giustificato (fara' presentazione via skype):
Prafulla Shantaram Katkar

II anno XXVII ciclo (10 min+5 min discussione):

Carfora Virginia

Relazioni attività di ricerca - III anno XXVI ciclo:

Barbara Arnò, XXVI ciclo
Docente guida: Prof. Alessandro Desideri

Experimental characterization of a human-plasmodial topoisomerase IB chimera

Plasmodium falciparum infection is one of the most frequent acquired red blood cell disease worldwide. The spread of multi-drug-resistant strains has increased the need to identify new molecular targets for antimalarial therapy. DNA topoisomerases may be considered possible candidates, due to their important role in cellular activities, including DNA replication. DNA topoisomerase I (Top1) relaxes supercoiled DNA by a transient DNA strand breakage, rotation, and religation. The human Top1 (hTop1) comprises a conserved protein clamp, tightly wrapped about the DNA duplex, formed by three protein domains and an extended coiled-coil linker domain that appropriately positions the C-terminal active site tyrosine against the core to form the catalytic pocket. The linker has been shown to be important in controlling the enzyme catalytic mechanism. Hence, after removal of the linker domain, hTop1 loses inter-domain communication, thus highlighting the importance of linker in controlling the religation and modulating CPT sensitivity (Chillemi et al., 2004; Stewart et al., 1999). The *Plasmodium falciparum* topoisomerase I (PfTop1) contains a longer linker, compared to the human homologue. This finding may open the way in exploiting its structural and functional properties in enzyme activity and drug sensitivity swapping the hTop1 linker domain with the corresponding one of PfTop1 (Arno et al., 2013b). Moreover, as the linker domain specifically affects the religation reaction, a pentacyclic-diquinoid synthetic compound, KuQ, has been designed and analyzed through activity assays and molecular docking, identifying crucial elements for this catalytic step of the hTop1 (Arno et al., 2013a; Coletti et al., 2012).

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 many genes. In every plate there are 86 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, 11 genes were downregulated and 5 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. We performed promoter activity assay, chromatin immunoprecipitation (ChIP) assay, real-time PCR and western blot analysis to confirm the direct regulation of CYGB by $\Delta Np63\alpha$ and to evaluate the biological role of this regulatory mechanism in keratinocytes.

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

Metabolic profiling of human CD4+ cells following treatment with Methotrexate and anti-TNF- α Infliximab

The autoimmune process in rheumatoid arthritis depends on activation of immune cells, which utilize intracellular kinases to respond to external stimuli such as cytokines, immune complexes, and antigens. CD4+ T cells comprise a large proportion of the inflammatory cells that invade the synovial tissue and may therefore be a cell type of pathogenic importance. Both methotrexate and infliximab are effective in the treatment of inflammatory arthritis, however the biological effects triggered by these treatments and the biochemical mechanisms underlining the cell response are still not fully understood. Thus, in this study the global metabolic changes associated with methotrexate or infliximab treatment of isolated human CD4+ T cells were examined using gas chromatography/mass spectrometry or liquid chromatography/mass spectrometry. In total 148 metabolites involved in selective pathways were found to be significantly altered. Overall the changes observed are likely to reflect the effort of CD4+ cells to increase the production of cellular reducing power to offset the cellular stress exerted by treatment. Importantly, analysis of the global metabolic changes associated with MTX or infliximab treatment of isolated human CD4+ T cells suggested that the toxicity associated with these agents is minimal when used at clinically relevant concentrations.

Dalya Elsaid Elshahat Hmada, XXVI ciclo
Docente guida: Prof. ssa Alessandra Gambacurta

Blood derived stem cells (BDSCs): Neural differentiation protocols for human therapy

Stem cells technology has evoked considerable excitement among people who interested in welfare of animals and human. Many people searching for tools helping in treating diseases and in vivo therapy. We know that all types of cells can be regenerated except neural cells of mammals such as don't regenerate, although the peripheral nervous tissue regenerate if neurillematic sheath allow the orientation of fiber. the importance of obtaining functional nerve cells is vital in neurodegenerative diseases. Neurodegenerative diseases are by definition as progressive chronic diseases characterized by a selective loss of neurons in areas, symmetric

motor , sensory , cognitive and membership of CNS, or loss / dysfunction of myelinated or non myelinated fibers in the PNS. Many neurodegenerative diseases including :Parkinson's, Alzheimer's, amyotrophic lateral sclerosis (ALS). Occur as result of neurodegenerative process. In this study using "neuron differentiation kits" has allowed me to achieve good results after maintaining and differentiation of blood derived stem cell (BDSCs) in specific medium : getting nerve cells from blood derived stem cells. obtaining the same results using substances non toxic and commonly used in human therapy. getting stem cells after deprogramming of peripheral adult blood with specific method. maintaining BDSCs in a specific medium. The BDSCs is able to form neurosphere And so, using differentiation protocols to achieve definite neurons in vitro. studying the molecular mechanisms that are needed to reprogram the BDSCs in neurosphere using immunocytochemistry , functional analysis such as RT- PCR , Western blotting assays.

Egidio Iorio, XXVI ciclo

Docente guida: Prof. Maurizio Paci

Tutor: Dr. Franca Podo/ Dr Rossella Canese

Effects of downmodulation of choline kinase and phosphatidylcholine-specific phospholipase C on the Magnetic Resonance Spectroscopy choline profile of epithelial ovarian cancer

The introduction of magnetic resonance spectroscopy (MRS) in cancer biology allowed the 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 and proposed these signals as a novel candidate hallmark for cancer. 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 and Cancer Res2010), 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, migration and alterations of the tCho profile (Granata et al, 2013 submitted). Pharmacological inhibition of PtdCho-plc induced a 30-to-40% reduction of PCho content and blocked in vitro cell proliferation in two EOC cell lines and in vivo tumor growth in a preclinical model in mice 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. The overall body of the presented data may help to elucidate aspects of EOC biology and provide a rational basis for further developing clinical non-invasive imaging methods suitable for ovary cancer diagnosis and follow-up.

Daniela Evangelista, XXVI ciclo

Docente guida: Prof.ssa Luciana Avigliano

2-Arachidonoylglycerol modulates human endothelial cell/leukocyte interactions by controlling selectin expression through CB₁ receptor

Accumulated evidence points to a key role for endocannabinoids in cell migration, and here we sought to characterize the role of these substances in early events that modulate communication between endothelial cells and leukocytes. We found that 2-arachidonoylglycerol (2-AG) was able to initiate and complete the leukocyte adhesion cascade by modulating the expression of selectins. A short exposure of primary human umbilical vein endothelial cells (HUVECs) to 2-AG was sufficient to prime them towards an activated state: within 1 hour of treatment, endothelial cells showed time-dependent plasma membrane expression of P- and E-selectins, which both trigger the initial steps (i.e., capture and rolling) of leukocyte adhesion. The effect of 2-AG was long lasting, because endothelial cells incubated with 2-

AG for 1 hour released the pro-inflammatory cytokine tumour necrosis factor- α (TNF- α) for up to 24 hours. Consistently, TNF- α -containing medium was able to promote leukocyte recruitment: human Jurkat T cells grown in conditioned medium derived from 2-AG-treated HUVECs showed enhanced L-selectin and P-selectin glycoprotein ligand-1 (PSGL1) expression, as well as increased efficiency of adhesion and trans-migration. In conclusion, our *in vitro* data indicate that 2-AG, by acting on endothelial cells, might indirectly promote leukocyte recruitment, thus representing a potential therapeutic target for treatment of diseases where impaired endothelium/leukocyte interactions take place.

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

Developmental factor IRF6 exhibits tumor suppressor activity in squamous cell carcinomas

IRF6 is a transcription factor that regulates craniofacial development. We have recently demonstrated that IRF6 is a component of a regulatory feedback loop that controls the proliferative potential of epidermal cells, being transcriptionally activated by Δ Np63 and inducing its proteasome-mediated downregulation. We supposed that IRF6 could be also involved in skin carcinogenesis. Therefore, we studied IRF6 expression in squamous cell carcinomas (SCCs), finding strong downregulation of IRF6 that correlated with methylation on a CpG island located in its promoter region. To identify the molecular mechanisms regulating IRF6 potential tumor suppressive activity, we performed ChIP-seq for IRF6 binding sites and gene expression profiling in primary human keratinocytes after siRNA-mediated IRF6 depletion. We found that IRF6 bound genomic regions are enriched in adhesion and proliferation genes; some of these were direct IRF6 targets. We also performed *in vitro* invasion assays showing that IRF6 downregulation promotes invasive behavior. Reintroduction of IRF6 in SCC cells strongly inhibits their growth. Our results highlight a novel function for IRF6 as tumor 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.

Emanuela Talamonti, XXVI ciclo
Docente Guida: Dott.ssa Filomena Fezza

Immunomodulatory role of Anandamide in the mononuclear phagocyte system

Anandamide (N-arachidonylethanolamine, AEA) is an endogenous arachidonic acid derivative and the most important lipid mediator of the endocannabinoid system (ECS), which is functionally involved in the regulation of several physiopathological processes, including neuroprotection and immune responses, acting mainly through cannabinoid receptors (CB1R and CB2R). Immune cells prominently express CB2R and possess the whole enzymatic machinery responsible for endocannabinoid metabolism. The present work investigates for the first time the role of AEA on the human mononuclear phagocyte system, that consists in monocytes, macrophages and dendritic cells (DC), in terms of their cell differentiation and polarization, activation status and inflammatory responses. We found that AEA affects the balance of classically activated M1-macrophages and of alternatively activated M2-macrophages, by switching M1 from a pro-inflammatory to an anti-inflammatory-like phenotype and by potentiating the anti-inflammatory responses of M2. Furthermore, we found that AEA inhibits NLRP3-inflammasome pathway in a CB2R-dependent manner, which is the prominent signalling pathway in human macrophages responsible for the production and release of the pro-inflammatory cytokine IL-1 β . Finally, AEA interfered with monocyte-derived DC maturation, affecting also DC-induced T cell activation. Thus, the ability of AEA to modulate the inflammatory responses of several immune cells of the mononuclear phagocyte system suggests that the ECS might hold therapeutic potential implication to target those chronic inflammatory diseases where such cells are involved, including cancer,

autoimmune and neurodegenerative diseases.

Tiziana Bisogno, XXVII ciclo

Docente guida: Prof. Finazzi Agrò

Inhibitors of the metabolism of endocannabinoids: potential therapeutic effects

The development of inhibitors of endocannabinoid (EC) metabolism is just starting to be considered as a promising source of new strategies to treat disorders that might benefit from a reduction or an enhancement in EC tone, such as hyperphagia in obese subjects and spasticity in multiple sclerosis, respectively. Three new compounds O-7458, O-7459 and O-7460 were synthesized and characterized in various enzymatic assays. O-7460 was identified as inhibitors of the biosynthesis of the EC 2-AG via diacylglycerol lipases (DAGL). When administered to mice, O-7460 (0–12 mg·kg⁻¹, i.p.) inhibited the intake of a high-fat diet over a 14 h observation period, and, subsequently, slightly but significantly reduced body weight. Moreover, the potential anti-spastic effect of inhibitors of anandamide degradation via fatty acid amide hydrolase (FAAH) was studied in an experimental autoimmune encephalomyelitis (EAE) model of MS induced in Biozzi ABH mice. Importantly, the therapeutic value of FAAH inhibitors was lost in FAAH-deficient mice. Therefore, EC metabolism inhibitors may be considered a novel pharmacological tool to investigate further the role played by the endocannabinoid system both *in vitro* and *in vivo* under physiological as well as pathological conditions.

Prafulla Katkar, XXVI cycle

Docente guida: Prof. Alessandro Desideri

Study of molecular mechanism of human DNA topoisomerase I and characterization of new novel topoisomerase IB inhibitors as a potent anticancer agent

The work mainly centered on the characterization of effects of transition metal complexes and natural compound on human DNA topoisomerase IB catalytic activity. Among the transition metal complex studied includes Cu(II), Zn(II), Co(II), Va(II), Gold(III) and natural compound thaspine. In the present work, we have investigated the ability of different oxindolimine metal complexes [Cu(isapn)]²⁺, [Zn(isapn)]⁺, [Cu(isaepy)]⁺, [Zn(isaepy)]²⁺ [Co(isapn)]²⁺ [Vo(isapn)]²⁺, Cyclometalated Gold(III) and natural alkaloid thaspine to interact with the human topoisomerase IB. The effect of all these compounds on the DNA relaxation activity and different catalytic cycle of topoisomerase IB has been analyzed by radioactive p³²-DNA labeling assay and molecular docking simulations. All compounds selected are able to inhibit the DNA relaxation and cleavage step of catalytic cycle. Cyclometalated Gold(III) is more efficient than the other metal complexes studied since it inhibits the topoisomerase I at lowest concentration of 10 μM. Gold (III) identified as catalytic inhibitor, since it inhibits the topoisomerase I cleavage reaction by not permitting the binding of the DNA substrate. Among the oxindolimine metal complex, [Cu (isapn)]²⁺ being more efficient than other metal compounds. Molecular docking simulation studies show that, the almost square planar geometry of the copper compound allows a direct coordination of the metal with amino acids of the enzyme at variance of the other metal compound which has a more tetrahedral geometry. Altogether, the data indicate that the different coordination geometry achieved by the transition metal ions has an important role in modulating their efficiency as topoisomerase I inhibitors.

Virginia Carfora, XVII ciclo

Tutor: Dott.ssa Giuseppina Giacinti- Istituto zooprofilattico Roma

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

Identification of enterotoxigenic strains of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin sensible Staphylococcus aureus (MSSA) isolated from bovine milk

Staphylococcus aureus is one of the most important foodborne pathogens, the use of antibiotics in veterinary practices could determine the selection of antibiotic-resistant clones of S. aureus, including Methicillin-resistant S. aureus (MRSA). Staphylococcus aureus produces a wide variety of toxins including staphylococcal enterotoxins (SEs): all possess superantigenic activity and are encoded by accessory genetic elements, including plasmids, prophages, pathogenicity islands, vSa genomic islands or by genes located next to the staphylococcal cassette chromosome (SCC) implicated in methicillin resistance. The aim of this research was to determine the prevalence of enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei, sej, sep, ser*) by Multiplex-PCR in MRSA and MSSA (methicillin sensible S.aureus) isolated from 75 individual bovine milk from seven different farms. Since the presence of SEs genes not always implies that the toxin is produced, the isolates positive to SEs genes were tested for the presence of staphylococcal enterotoxins (SEA-SED) by using the reversed passive latex agglutination (RPLA) method. Of 75 isolates examined, 20 (22,7 %) were found to be positive for SEs genes, while SEs were observed in only one strain. Seh was most commonly detected in MRSA isolates.

- Venerdì' 25 Ottobre ore 9.30, Aula D29: 10 min+5 min discussione

III anno XXVI ciclo:

Hanno richiesto la proroga di 1 anno:

Mara Mancini
Rossella Loria

II anno XXVII ciclo:

Andrea Farrotti
Arianna Giacobbe
Ramona Palombo
Sebastiano Cefalù
Sara Vieira
Sara Adanti

I anno XXVIII ciclo:

Marco Pieraccioli
Carla Regina
Gianluca Noce
Arianna Ceccarelli
Anna Giulia Sanarico
Daniela Gnani
Zhenxing Wang

Relazioni attività di ricerca - III anno XXVI ciclo:

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

Non-Coding Ultraconserved RNA: Role of T-UC 291 in human epidermal keratinocytes

Transcribed-ultraconserved regions (T-UCRs) are a family of non-coding RNA (ncRNAs) that are transcribed from regions that exhibit extremely high conservation between the orthologous regions of human, rat and mouse genomes. In the human genomes, T-UCRs are found to overlap with exons of genes involved in RNA splicing or are located within host gene introns or in close proximity of genes that are involved in transcription and development regulation. While ultraconserved sequences appear to act as regulators of gene expression during development, very little is currently understood about the mechanism by which they act. Here, we investigated the role of T-UC 291 in human epidermal keratinocyte differentiation. By microarray profiling of T-UCRs levels modulated during HEK293T differentiation, we selected T-UC 291 as one of the most up-regulated ncRNAs. T-UC 291 is a part of an intron of the host protein-coding gene, C10orf11. We showed that these T-UC is predominantly nuclear and we provide evidence that T-UC 291 down-regulation in HEK293T cells is sufficient to induce an increase of proliferation rate and delay in vitro keratinocytes differentiation. Our preliminary data suggest that T-UC 291 could have an important role in the regulation of cell proliferation and differentiation and the future plan is to determine the mechanism by which it acts.

Rossella Loria, XXVI ciclo
Tutor: Dott.ssa Rita Falcioni
Docente guida: Dott.ssa Eleonora Candi

Sema6A and Mical-1 sustain cell growth and survival of BRAF^{V600E} mutated human melanomas

The majority of cutaneous melanomas show activating mutations in the *BRAF* or *NRAS* proto-oncogenes, leading to constitutive activation of signal transduction pathways involved in the control of cell survival and proliferation. Genetic changes affecting *NRAS* and *BRAF* behave as driver mutations and occur in ~20% and ~70% of tumors, respectively. BRAF^{V600E}-specific and MEK-specific inhibitors have shown significant impact on progression-free and overall survival in advanced melanoma. However, primary and secondary resistance mechanisms restrict the efficacy of these target-specific drugs, suggesting that additional therapeutic targets need to be identified, first of all in the highly frequent BRAF-mutant melanomas. To this end, we used whole genome microarray analysis to identify potential candidate genes with differential expression in BRAF- vs NRAS-mutant neoplastic cells. We selected, for comparison, BRAF-mutant and NRAS-mutant cell lines, as well as a peculiar model based on melanoma clones, isolated from a single tumor characterized by mutually exclusive expression of activated BRAF and activated NRAS in different cells. This effort led us to identify two genes, SEMA6A and Mical-1, highly expressed in BRAF-mutant neoplastic cells. Real-time PCR, Western blot analysis on cell lines and immunohistochemistry on tissue sections from BRAF and NRAS-mutant tumors confirmed the preferential expression of SEMA6A and Mical-1 in BRAF-mutant melanomas. SEMA6A is a member of the semaphorin family, and it complexes with the plexins to regulate actin cytoskeleton, motility and cell proliferation. By specific RNA-interference experiments, we found that SEMA6A depletion causes cytoskeletal remodeling, loss of stress fibers, generation of actin-rich protrusion, and cell death by anoikis. Mical-1 can interact with SEMA6A, but its major role is to bind the phosphorylation site of NDR (a kinase that promotes apoptosis), thus inhibiting NDR activation by the upstream kinase MST-1. By specific Mical-1 RNA-interference, we found that Mical-1 depletion restores MST-1-dependent NDR phosphorylation and promotes apoptosis. Overall, our data suggest that SEMA6A and Mical-1 are new potential therapeutic targets in BRAF-mutant melanomas.

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 nowadays a useful tool that, coupled with experimental data, can describe at atomic level many different biological processes. Sampling problems, typical of all-atoms (AA) simulations, can be overcome by using coarse-grained (CG) force-fields, that allow us to perform millisecond simulations on very complex systems, at the price of a loss in the resolution. By employing both AA and CG force-fields, we are carrying on two main research projects. The first is focused on the effects of different pathogenic mutations on the structure and dynamics of Ras-MAPK pathway proteins, such as R-RAS and SHP-2. In particular, our simulations on R-RAS revealed the possible molecular mechanism related to the higher rate of GDP dissociation in the V55M mutant observed by fluorescence experiments. Currently, we are performing replica exchange MD (REMD) simulations on SHP-2 protein in order to characterize the role of an α -helix in the catalytic process. The other area of interest comprises the study of the interaction between antimicrobial peptides (AMPs) and lipid bilayers. AMPs are a class of molecules able to kill bacteria by inducing the formation of pores in their membranes. Knowledge of peptide orientation in the membrane is essential to discriminate between different pore formation mechanisms of AMPs. In order to predict this information, we employed computational methods such as the “minimum-bias” approach or potential of mean force (PMF) calculations, with either AA or CG force-fields. In particular, we assessed the reliability of these methods, by applying them to a very stringent test case. NMR experiments demonstrated that the amphipathic LAH4 AMP, which contains four His residues, is bound to the membrane surface at acidic pH, while it assumes a transmembrane orientation at neutral pH. Our results show that computational approaches are able to reliably predict peptide orientation in membranes.

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.

In addition, MTSS1 expression is down-regulated in metastatic breast carcinoma cell line after siRNA-mediated depletion of p63 or treatment with TGF β , a pro-invasive and pro-metastatic factor. We also found that MTSS1 and TAp63 transcriptional levels are correlated both in breast and bladder tumor cell lines. These data suggest that TAp63-MTSS1 axis might be important in the regulation of the migration and invasiveness properties of cancer cells.

Ramona Palombo, XXVII ciclo
Tutor: Dr. Alessandro Terrinoni
Docente guida: Prof. Luciana Avigliano

Metabolomic study of human epidermal keratinocytes after luteolin 7-glucoside treatment

Luteolin is the most common bioflavonoid present in edible plants (fruit and vegetables), and in plants used for traditional medicine. It is often found in many of the same foods that contain apigenin. According recent literature, luteolin is involved in multiple biological effects, like anti-inflammation, anti-allergic, anti-cancer, and also in redox regulating activities, since it can be considered like a scavenger molecule towards free radicals. Some of its effects like the anticancer activity are linked to pro-apoptotic and anti-proliferative (cytostatic) effects. It would be interesting to study luteolin biology in an easy accessible organ like the skin, for this purpose we decided to use an epidermal keratinocytes proliferating/differentiating system. After 24-48h of treatment with 20 μ M of luteolin (7-glucoside), human keratinocytes shows changes in cell cycle progression, with accumulation in G1 phase. Furthermore the treatment leads to the expression of keratinocyte differentiation markers, while a prolonged exposure to this flavone seems to have effect also in the senescence process. Studies on the metabolic effects of the flavone, demonstrate a strong depressive effect on the cell energy production and a change in the pattern of intracellular lipids produced.

Sebastiano Cefalù, XXVII ciclo
Docente guida: Prof.ssa Eleonora Candi

Investigation into the possible roles of TAp63g 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 vitro and in vivo.

Sara Vieira, XXVII ciclo

Docente guida: Prof. Alessandro Desideri

Interaction of human DNA topoisomerase IB wild type with Berberine chloride compound and its derivatives

DNA topoisomerases play a crucial 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 formation of a transient covalent enzyme-DNA intermediate. DNA topoisomerases are classified into type I and type II and first type is the only target of camptothecin (CPT), a natural plant alkaloid. Topoisomerases IB are anticancer and antimicrobial targets whose inhibition by several natural and synthetic compounds has been documented over the last three decades. Human DNA Topoisomerase IB (hTopIB) is constituted by four domains: N-terminal, core, linker and C-terminal domain. Berberine chloride derivatives are new compound that showed inhibition of hTopIB activity. During my second year, I am analyzing the mechanism of these compounds on relaxation activity and on each step of the catalytic cycle of hTopIB. The preliminary results demonstrated that those compounds inhibit the relaxation of supercoiled DNA and pre-incubation of hTopIB with the compounds before adding DNA substrate increases the inhibitor effect, for all the derivatives. Cleavage and religation reactions will be assayed separately in order to determine which step of enzyme catalytic activity is disturbed in presence of them.

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 identified 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 new data show that the pharmacological inhibition of TCTP by Dihydroartemisinin reduces the intracellular TCTP levels and cell growth of human breast cancer cell lines and improves conventional chemotherapy treatments.

Relazioni attività di ricerca - I anno XXVIII ciclo:

Marco Pieraccioli, XXVIII ciclo
Tutor: Dr. Giuseppe Raschellà
Docente Guida: Prof. Gerry Melino

Mechanism(s) of control and functional role of ZNF281 in DNA damage response

ZNF281 is a zinc finger type transcription repressor that plays a role in the regulation of embryonic stem cells (ESCs) differentiation and acts by mediating autorepression of NANOG in ESCs. Previous data demonstrated that ZNF281 is not required for establishment and maintenance of ESCs pluripotency. As a transcriptional regulator, ZNF281 represses the transcription of a number of genes including GAST, ODC1 and VIM by binding the G-rich boxes in the enhancer region of these genes. By using p53 and p73 inducible SaOS2 cells, I demonstrated that both p53 and p73 were able to induce the expression of ZNF281. Accordingly, after DNA damage induction by several chemical agents (doxorubicin, etoposide, camptothecin), ZNF281 expression increased in wt p53 but not in null p53 cells. In keeping with a p53-dependent control, when p53 was silenced by siRNA, the increase of ZNF281 after DNA damage was abolished in wt p53 cells. To understand whether transcription activation was at least in part responsible for the p53-dependent induction of ZNF281, *in silico* analysis of proximal region of the human ZNF281 promoter showed two potential p53 binding sites. I cloned the proximal region of the ZNF281 promoter into a reporter vector to carry out functional assays. Furthermore, to investigate the role of ZNF281 in the DNA damage response pathway I used a RT² profiler PCR Array which allows to measure the expression of 84 DNA damage related genes. I silenced ZNF281 in U2OS cells and after 24 hours I induced DNA damage by etoposide treatment. After additional 24 hours I carry out the array experiment. Several genes implicated in the DNA damage response were found down-regulated in ZNF281 silenced cells after DNA damage. The majority of these down-regulations were confirmed in 2 independent experiments by qPCR analysis. These preliminary results suggest that ZNF281 participates to the DNA damage response by modulating the expression of relevant genes in the pathway.

Carla Regina, XXVIII ciclo
Docente Guida: Prof.ssa Eleonora Candi, prof. Sergio Bernardini

Dissecting the role of p63 in breast cancer: looking for (TA)p63a's interactors

p63 is the most ancient member of p53 family of gene, in which is included, besides p53, also p73. p63, like the other two members, uses an alternative promoter at the 5' end of the gene to allow the expression of two different N-terminal isoforms, one containing the N-terminal transactivation domain (TA isoform) and an N-terminal truncated isoform (Δ N isoform) that lacks this domain. Moreover, the C-terminal sequence undergoes alternative splicing that gives rise to a wide range of TA and Δ N isoforms with different C-terminal organization (alpha, beta and gamma isoforms). p63 containing the transactivation domain (TAp63) and amino-deleted p63 isoforms (DNp63) exert distinct (often opposite) functions on stemness, cycle arrest, mobility and invasion (epithelial-mesenchymal transition, EMT) and senescence. It is known that TAp63 induces cell death and cell cycle arrest with tumor suppressor features, while DNp63 exerts oncogenic properties and is generally overexpressed in cancer. The aim of our work is to dissect (TA)p63's contribution to breast carcinogenesis by looking for its possible interactors in this cellular system. As a consequence of this, we first performed an expression screening of p63 isoforms in several breast cancer cell lines. Besides, data obtained from a yeast two hybrid assay (made by the Hybrigenics bio-pharmaceutical company and performed towards the C-terminal of the protein: aa 444-680), show several possible interactors of the protein, in particular of alfa-isoforms. Through immunoprecipitation assay, made in HEK293T cells, we found ZNF451 (multi-zinc finger protein) as a both TA and DNp63a's interactor. Our goal is now trying to understand p63 interaction domain and the importance of this interaction in breast cancer.

Gianluca Noce, XXVIII ciclo
Docente guida: Prof. Sergio Bernardini

Dosaggio degli acidi grassi poliinsaturi plasmatici e di membrana eritrocitaria nei pazienti emodializzati e nella popolazione generale

Numerosi studi epidemiologici hanno dimostrato che la mortalità e la morbilità cardiovascolare in pazienti emodializzati è aumentata rispetto alla popolazione generale. E' ormai accertato che gli acidi grassi poliinsaturi (PUFA) riducono il rischio di malattia cardiovascolare (CVD) nella popolazione generale in virtù della loro importante funzione antiaritmica e di regolazione dei livelli dei lipidi ematici.

Lo scopo del nostro lavoro è stato quello di determinare la composizione dei PUFA plasmatici totali e di membrana in un gruppo di pazienti caucasici in emodialisi (HD) e in un gruppo di soggetti sani (soggetti di controllo). Inoltre è stata valutata la relazione tra gli acidi grassi omega-6 ed omega-3, acido arachidonico (ARA)\acido eicosapentanoico (EPA) e acido arachidonico (ARA)\acido docosaesanoico (DHA), e le possibili differenze in concentrazione nei PUFA tra i due gruppi studiati. I PUFA plasmatici totali e di membrana eritrocitaria sono stati analizzati mediante gas-cromatografia accoppiata ad uno spettrometro di massa dopo estrazione rispettivamente da plasma e sangue intero.

Nel nostro lavoro è stata osservata una diversa composizione dei PUFA totali e di membrana tra la popolazione generale e i soggetti emodializzati. Monitorando le concentrazioni dei PUFA di membrana dei globuli rossi è possibile predire il rischio cardiovascolare nei pazienti emodializzati. Pertanto una dietoterapia mirata, finalizzata ad aumentare i livelli di PUFA omega-3 e ad ottimizzare il rapporto tra omega-6 e omega-3, potrebbe esercitare un'azione protettiva cardiovascolare.

Arianna Ceccarelli, XXVIII ciclo
Docente Guida: Prof. Nicola Rosato

"Study of the dynamic structure and of the function of multimeric and monomeric proteins using fluorescence spectroscopy"

The TNF receptor-associated factor 2 (TRAF2) is a member of the Tumor Necrosis Factor (TNF) receptor associated factor (TRAF) protein family which associate with, and mediate the signal transduction from members of the TNF receptor superfamily.

We have determined the secondary structure content of this protein using Circular Dichroism and our results parallel the crystallographic data, confirming that even in solution TRAF2 contains a high percentage of β -sheet and a smaller contribution of α helices. The tertiary structure was analyzed monitoring the intrinsic fluorescence of the tryptophan residues with both steady-state and time-resolved fluorescence. Both tryptophylic result to be fully buried with lifetime of 0.55 ns and 5.9 ns.

We also characterized the behavior of the protein under denaturing condition such as high pressure, chemical denaturing agents (guanidine) and temperature, performing CD spectra and steady-state fluorescence spectra. The experiments were carried on at different concentrations of the protein to study aggregation equilibrium. TRAF2 resulted to be quite stable. However, the steady-state fluorescence spectra obtained at increasing temperature suggest that the protein molecules are prone to associate at high temperature. Light scattering experiments confirmed such findings. We also studied the formation of a complex comprising TRAF2 and glutathione S-transferase pi 1 (GSTP1). In particular we monitored the interaction of dansylated TRAF2 with increasing amounts of GSTP1 in the presence and in the absence of 1mM GSH. The result yielded a binding curve which was fitted to a mathematical model, obtaining a dissociation constant of $0.3 \pm 0.02 \mu\text{M}$ and $4.5 \mu\text{M}$ in the presence and in the absence of GSH, respectively. Such results demonstrated that the presence of GSH reduce the affinity of GSTP1 for TRAF2.

Anna Giulia Sanarico, XXVIII ciclo
Docente guida: Dott.ssa Francesca Bernassola

Unveiling the oncogenic role of the E3 ubiquitin ligase WWP1 in the pathogenesis of Acute Myeloid Leukaemia

Acute Myeloid Leukaemia is a genetically heterogeneous aggressive disorder resulting from accumulation of multiple karyotypic, genetic or epigenetic changes that lead to impaired differentiation and increase self-renewal ability of hematopoietic progenitors. The whole spectrum of abnormalities underlying AML pathogenesis has not been fully elucidated. The aim of the project is to find a possible involvement of the E3 ubiquitin ligase, WWP1, in the pathogenic progress of the leukaemia, since it is a potential oncogene found deregulated in solid human cancers and its overexpression has been associated with increased solid cancer cell proliferation and survival. In our experiments we found increased levels of WWP1 mRNA in leukemic samples from patients as compared to healthy donor controls. Also the analysis of the expression levels of WWP1 in AML and APL cell lines showed elevated WWP1 protein levels as compared to the control cells. Experiments of knockdown on cell lines supported the hypothesis that WWP1 is involved in deregulation of cell cycle, apoptosis and differentiation of AML cells. Instead, overexpression of WWP1 in hematopoietic stem progenitors cells did not affect their self-renewal and differentiation capabilities. In conclusion, our data demonstrate that WWP1 is aberrantly expressed in leukaemia cells and indicate that increased levels of WWP1 may contribute to survival and proliferation of AML blasts.

Daniela Gnani, XXVIII ciclo

Tutor: Dott.ssa Anna Alisi – Ospedale Pediatrico Bambino Gesù di Roma

Docente guida: Prof.ssa Isabella Savini

In vitro NAFLD lead to hepatic stellate cells activation and epithelial-to-mesenchymal transition

NAFLD is a multifactorial condition, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) with or without liver fibrosis. Coordinated action of several factors, including oxidative stress, inflammation, and increased gut bacterial endotoxin, can play a role in the progression from simple steatosis to NASH. In the initial phase of fibrosis, hepatocytes play an important role in the production of pro-fibrogenic factors, triggering the trans-differentiation of hepatic stellate cells (HSCs), which can be identified as epithelial-to-mesenchymal transition (EMT).

In order to study this complex network of interactions often leading to the development of fibrosis, we studied *in vitro* the direct effect of LPS and the indirect effect of molecules released by fatty and redox imbalanced-hepatocytes (HepG2 cells) on the activation of HSCs (Lx-2 cells).

To this aim, we treated Lx-2 cells with LPS and we found a switch towards a activated inflammatory phenotype rather than EMT. Then, using a NAFLD *in vitro* model of HepG2 cells with high levels of FFA and H₂O₂, we reported that the conditioned medium from stimulated HepG2 was able to drive the activation of Lx-2 cells. Therefore it is conceivable believe that combination of the indirect effects of FFA and H₂O₂ with the direct LPS treatment of Lx-2 may reproduce the EMT events occurring during NAFLD.

The role of the Polycomb Group protein Enhancer of Zeste Homolog 2 (EZH2) that controls epigenetic silencing of specific genes and/or microRNAs in NAFLD is still unknown. Our recent preliminary data, demonstrate that EZH2 expression/activity is down-regulated *in vivo* and *in vitro* NAFLD and that the inhibition of EZH2 inversely correlated with lipid accumulation, expression of pro-inflammatory markers and specific microRNAs. Therefore, we also investigated in our models the potential role of EZH2 in the activation and EMT of HSCs.

Zhenxing Wang, XXVIII ciclo

Docente guida: Prof. Alessandro Desideri

Investigation of the human Topoisomerase IB Arg634Ala mutation

Human topoisomerase 1B, the unique target of the natural anticancer compound camptothecin, catalyzes the unwinding of supercoiled DNA by introducing transient single strand nicks and providing covalent

protein-DNA adducts. The functional properties and the drug reactivity of the single Arg634Ala mutant have been investigated in comparison to the wild type enzyme. The mutant is characterized by an identical relaxation and cleavage rate but it displays resistance to camptothecin as indicated by a viability assay of the yeast cells transformed with the mutant protein. The mutant also displays a very fast religation rate that is only partially reduced by the presence of the drug, suggesting that this is the main reason for its resistance. A comparative analysis of the structural-dynamical properties of the native and mutant proteins by molecular dynamics simulation is also conducted to confirm the motion correlation of Arg634 mutation. These results indicate that the loss of motion correlation and the drug resistance are two strongly correlated events.