Expression, regulation and intracellular signalling cascades triggered by Formyl-peptide receptor 2

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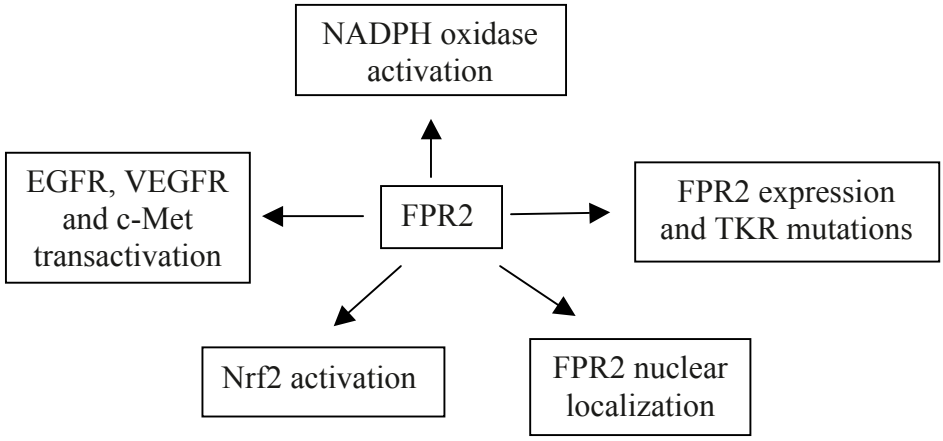
The aim of our studies is to establish new concepts on function and regulation of Formyl-peptide receptor 2 (FPR2), which could have an impact on development of new diagnostic and therapeutic strategies. FPR2 belongs to the GPCR family and is implicated in many pathophysiological processes. It is activated by an array of agonists, which include structurally unrelated lipids and peptide/proteins, resulting in potent pro- or anti-inflammatory responses and in NADPH oxidase-dependent ROS generation, in a ligand-specific fashion (1). Our results show: i) that FPR2 stimulation triggers transactivation of Tyrosine Kinase Receptors EGFR, c-Met and VEGFR and the activation of the relative intracellular signalling cascades. The molecular mechanism of this cross-talk requires FPR2-dependent NADPH oxidase activation (2, 3); ii) a correlation between FPR2 expression in biopsies of human lung cancer resistant to therapy with VEGFR or EGFR inhibitors and EGFR mutations; iii) the nuclear translocation of the transcriptional factor Nrf2 in FPR2-stimulated cells. Nrf2 is crucial in maintaining cellular homeostasis, upon the exposure of cells to chemical or oxidative stress, through its ability to regulate the basal and inducible expression of many antioxidant proteins, detoxification enzymes and xenobiotic transporters. Aberrant expression and/or function of Nrf2 is associated with many pathologies; iv) that FPR2 is also localized onto nuclear membrane eliciting some selective signalling cascades.



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Analysis of the interaction between gastrokine1 and β-amyloid peptide: anti-amylogenic property of the protein.

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Aim: Gastrokine 1 (GKN1) is a stomach-specific protein important for maintaining the physiological function of the gastric mucosa. GKN1 is characterized by the presence of a BRICHOS domain consisting of about 100 amino acids found in several unrelated proteins associated with major human diseases like BRI2, related to familial British and Danish dementia; chondromodulin-I (ChM-I), linked to chondrosarcoma; surfactant protein C (SP-C), associated with respiratory distress syndrome; and gastrokines, linked to gastric cancer. Literature data show that recombinant BRICHOS domains from Bri2 and SP-C precursor (proSP-C) prevent fibril formation of amyloid-beta peptide (Aβ) that is the major component of extracellular amyloid deposits in Alzheimer's disease. Aβ derives from the partial hydrolysis of the amyloid precursor protein (APP) catalyzed by β-and γ-secretase. The hydrolysis produces amyloid peptides of 40 or 42 amino acid residues. Here we investigate the interaction between recombinant GKN1 (rGKN1) and Aβ (1-40).
Methods: Aβ was incubated in the presence and absence of rGKN1 and chicken cystatin, as negative control, at 5:1 molar ratio. Samples were then analyzed by SDS-PAGE. The ability of GKN1 to dissociate already formed fibrils was also investigated using Thioflavine T binding assay. The formation of a GKN1/Aβ complex was evaluated by a Native-PAGE and mass spectrometry analyses and finally surface plasmon resonance were performed to characterize this interaction.
Results: rGKN1 prevented amyloid aggregation and fibril formation by inhibiting Aβ (1-40) polymerisation. This result was also confirmed by Thioflavin T binding experiments. Native-PAGE and mass spectrometry analyses showed the formation of rGKN1/Aβ complex. BIAcore kinetics of rGKN1/Aβ interaction led to calculate a dissociation constant (K_D) of 34 μM.
Conclusions: These preliminary data strongly indicated that GKN1 posses anti-amyloid activity. These results suggest that GKN1 might play a role as chaperone toward unfolded amyloid-like peptides thus preventing their aggregation.

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Molecular basis of cell function

S. Bonatti, M. Mallardo, G. Mottola, G. Caporaso

- Bonatti, Caporaso: *Rescue of proper localization of mutated human receptors and transporters*
A number of human hereditary pathologies are linked to loss of function of mutated transmembrane proteins (receptor, channel, pump or transporter) that fail to attain native configuration and are retained in the endoplasmic reticulum. We have recently shown that i) the retention of a mutant Frizzled4 (Fz4) associated with a dominant form of human vitreoretinopathy (Lemma *et al.*, 2013, *Sci Rep*) is due to a disorder-to-order transition in the cytosolic tail; ii) the cytosolic chaperone a-crystallin B is able to rescue folding and compartmentalization of the same Fz4 mutant and of the mutant copper transporter ATP7B-H1069Q, responsible for the most common form of Wilson disease in the Caucasian population (D'Agostino *et al*., 2013, *J Cell Sci*).
Collaboration: drs. R. Polishchuk, TIGEM, Naples and M. Stornaiuolo, visiting scientist.

- Mallardo, Caporaso: *MicroRNAs involved in human diseases*
The discovery of microRNAs has uncovered a new level of complexity for every biological process. Through the modulation of translation, microRNAs alter the basal state of cells and the outcome of stimulatory events. microRNAs network effects on human diseases is just starting to be dissected.
Our lab demonstrated that miR-155 is potentially involved in the aetiology of Burkitt Lymphoma (NAR 2008) and results up-regulated in primary and secondary glioblastoma promoting tumour growth by inhibiting GABA receptors (*Int. J. oncol.* 2012).
Collaboration: prof. Capasso (Nephrology, SUN) in understanding miRNA's role in onset of hypertension.

- Mottola, Bonatti: *Phagocytosis and phagosomes maturation: from the model organism planaria to humans phagocytic cells*
We are interested in the mechanisms that phagocytic cells exploit in order to internalize and eliminate foreign organisms and that bacterial pathogens hijack to survive and replicate. Specifically, we are studying the role of the *Coxiella burnetii* lipopolysaccharides on phagocytosis and phagosomes maturation in macrophages (Mottola *et al.*, 2012, *Cell Host & Microbe*). Additionally, we are establishing planarian worms as model organism for the identification of novel conserved molecules that are involved in the phagocytic activities of immunity cells (Mottola G. *et al.,* submitted).
Collaboration: Dr E. Ghigo and Prof. J.L. Mege, Team Infections, Gender and Pregnancy, URMITE, Faculté de Médecine, Marseille, France.

Role of NCOA4 protein in DNA replication control and tumourigenesis

Roberto Bellelli, Maria Domenica Castellone, Anna Maria Cirafici, Giorgia Federico, Roberto Limongello, Massimo Santoro, Francesca Carlomagno

DNA replication origin firing is tightly controlled to avoid activation of too many origins simultaneously, to prevent transcription/replication conflicts and to block DNA replication in case of DNA damage. Unscheduled replication might lead to genomic instability and cancer.

NCOA4 encodes a transcriptional coactivator of nuclear hormone receptors that, in thyroid and lung cancer, undergoes recombination with *RET*, generating RET/PTC3 oncogene. By combining studies in *Xenopus l.* egg extracts and *NCOA4 null* mouse embryonic fibroblasts (MEFs), we have demonstrated that NCOA4 is essential to set the number of active DNA replication origins, therefore preventing replication stress and cellular senescence. Indeed, NCOA4 forms a complex with MCM2-7 helicase. In egg extracts, NCOA4 immunodepletion increases active replication origins number, while excess of NCOA4 inhibits DNA replication by obstructing MCM2-7 helicase activity. *NCOA4 null* MEFs display reduced inter-origin distance and replication stress signs and undergo premature senescence.

We are interested in exploring the role that NCOA4 protein has in:
1. origin selection in transcriptionally active chromatin by analysing:
i) NCOA4 binding sites on whole genome
ii) the effect of oestrogen stimulation on the process of origin selection
iii) the role of NCOA4 in transcription-dependent regulation of DNA replication origin selection
2. the regulation of origins activation during DNA Damage Response (DDR) by studying:
i) the effect of NCOA4 knock down on cell viability upon genotoxic stress
ii) the DNA damage sensitive phenotype in *NCOA4 null* mice
iii) NCOA4 protein direct involvement in DDR
3. promoting carcinogenesis when fused to RET protein in RET/PTC3 fusion by investigating the role of *NCOA4* gene disruption in mice expressing RET/PTC3 rearrangement in thyroid follicular cells and bronchial epithelium

We believe that these studies will help understanding the role of NCOA4 in controlling genomic stability acting as a barrier for tumourigenesis.

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Bellelli et al. NCOA4 transcriptional coactivator inhibits activation of DNA replication origins. Submitted

Molecular basis for multifunctional roles of the KRAB zinc finger protein ZNF224

Elena Cesaro, Giorgia Montano, Paola Izzo, Paola Costanzo

Our research interests focus on understanding molecular mechanism and functional role of the KRAB-zinc finger protein ZNF224 in transcriptional regulation. This protein represents a useful system to analyze *in vivo*, at gene-specific loci, the recruitment and the repression mechanism of KRAB-ZFP, being one of the few KRAB-ZFPs whose target gene has been identified. Previously, we analyzed the KAP1-dependent molecular mechanism of transcriptional repression mediated by ZNF224 and identified the protein arginine-methyltransferase PRMT5 as a component of the ZNF224 repression complex, thus providing novel insight into KRAB-ZFP epigenetic mechanisms (1). Our recent findings indicate that KAP1 is a novel substrate for PRMT5 methylation. Our future efforts will be directed to analyze the effects of KAP1 modifications on the function of KRAB/KAP1 complex in regulation of gene expression.
In addition to its role of transcriptional repressor, ZNF224 acts as a transcriptional co-factor of the Wilms' tumor protein 1 (WT1) (2). ZNF224 acts in fine tuning of WT1-dependent control of gene expression, enhancing the WT1-mediated transcriptional activation of proapoptotic genes and suppressing WT1 transactivation of antiapoptotic genes in the chronic myelogenous leukaemia (CML) K562 cell line. Furthermore, cytosine arabinoside (ara-C) and imatinib increase ZNF224 expression; this induction increases susceptibility to apoptosis of K562 cells (3). Our findings establish a role for ZNF224 in apoptotic processes in CML and may have implications for cancer chemotherapy strategies.
Instead, our recent findings propose a proliferative role for ZNF224 in Chronic Lymphocitic Leukemia (CLL), likely through the transcriptional modulation of cyclin D3 expression. Moreover, we observed a significant correlation between the number of lymphocytes B, ZNF224 expression and the expression of cyclin D3 in 80 CLL samples.
A better definition of the molecular mechanism underlying ZNF224 mediated transcriptional regulation, through the identification of new interacting protein and target genes, will improve our understanding of its role in different cellular pathway.

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Evaluation of cytotoxic and pro-apoptotic effects of 7-dehydrocholesterol on melanoma cell line

Monica Gelzo[1], Giuseppina Granato[1], Francesco Albano[2], Alessandro Arcucci[2], Antonio Dello Russo[1], Emmanuele De Vendittis[1], Maria Rosaria Ruocco[1], Gaetano Corso[3]

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Contents

Ultraviolet radiation is the main cause of skin cancers, and melanoma is the most serious form of tumor. Surgery is standard treatment for localized melanoma, while there is no therapy for advanced-stage of melanoma and its metastasis, because of the high resistance of melanoma cells to various anticancer therapies[1]. Human skin is an important metabolic organ in which occurs photo-induced synthesis of vitamin D3 from 7-dehydrocholesterol (7-DHC)[2]. The 7-DHC, the precursor of cholesterol biosynthesis, is highly reactive and easily modifiable to produce 7-DHC-derived compounds. The intracellular levels of 7-DHC or its derivatives can have deleterious effects on cellular functionality and viability[3].

In this study we evaluated the effect on A2058 melanoma cell line by 7-DHC as such and for this aim much care to minimize 7-DHC modifications was used. We found that from 12 to 72 hours of treatment 82-86 % of 7-DHC entered into the cells, and the levels of 7-DHC-derivative compounds were not significant. At same time ROS production was significantly increased already after 2 hours and, after 24 hours, a reduction of cell viability was observed. Indeed, after 48-72 hours a pro-apoptotic effect of 7-DHC was detected. The increase of ROS levels, associated to a decrease of Bcl-2/Bax ratio, a reduction of mitochondrial membrane potential and an increase of AIF protein levels could explain the mechanism through which 7-DHC exerts its pro-apoptotic effect. This is the first report in which the biological effects found in A2058 melanoma cells are mainly attributable to 7-DHC as such.

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Modification of the accessibility of the Hu-ALB promoter in cell lines transfected with the HNF1β/LFB3 transcription factor.

Michele Olivieri, Alessia Ingenito, Vittoriana De Pasquale, Giovanna D'Agostino, Vincenzo De Simone

The human albumin gene is located in a complex chromosomal environment, the albumin/α-fetoprotein (Alb/AFP) gene cluster. Alb and AFP genes are developmentally regulated: AFP gene expression starts very early during development and is switched-off at birth, when Alb expression "takes over" and remains steadily active in the adult liver cells.

A pivotal role in the transcriptional regulation of these two genes is played by the HNF1α and β transcription factors, whose binding sites are located in both promoters, as well as in the Alb and in the multiple AFP enhancer regions. These two TF are almost identical in the N-terminal DNA binding region, but markedly different in the C-terminal activation domain. Another remarkable difference is that HNF1β (originally named LFB3) is expressed much earlier than HNF1α and much before liver differentiation occurs.

This last observation leads to the hypothesis that HNF1β-LFB3 may act primarily as a chromatin modifier, opening up the relevant regulatory regions and paving the way for later HNF1α action. Therefore, we decided to investigate if HNF1β-LFB3 ectopic expression could influence the accessibility of Alb and AFP regulatory regions in cell lines transfected with HNF1β-LFB3 expression vectors.

To this purpose, we have employed the FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) technique (1) on human and mouse cell lines transfected with a variety of HNF1 expression vectors. The accessibility of the Alb and AFP promoters has been assessed by conventional and qPCR quantization of the promoter sequences in the FAIRE DNA fraction.

Our results indicate that only HNF1β-LFB3 expression, but not HNF1α- or a C-terminal truncated version of HNF1β-LFB3, leads to a significant increase of Alb promoter sequences in HeLa cells FAIRE extracts. Experiments with different cell lines, as well as with the TACh complementary technique (2) are currently in progress.

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How (and why) to expose high school students to the emerging concepts at the frontiers of molecular biology: five years experience with the "Eureka" project.

L. De Magistris, M. Olivieri, L. Ippolito, F. Vuolo, F. Bergantino, F. De Simone, M. Piedimonte, V. De Simone

AIMS OF THE PROJECT: To arouse the interest of students in molecular biology and biotechnology and their impact in our society; to stimulate the interest for scientific disciplines in high schools oriented to humanistic studies.

METHODOLOGY: Simplified experiments addressing emerging topics in molecular biology carried out at school by a group of 30 students, assisted by our research team. The experimental phase consists in four laboratory and one computer sessions, preceded by a single theoretical lesson. A group of 4-6 students is selected to presents the results of the experiments in a plenary lecture to the whole institute followed by interdisciplinary discussions involving the students, the teaching staff, and academic guests.

TOPICS: In the last five years we have addressed the following topics:
• *Genome*: Analysis of the student's own DNA for a polymorphism of the HFE gene.
• *Metagenome*: Identification of bacteria from marine samples by 16s rDNA sequencing.
• *Phylogenesis*: Building a phylogenetic tree by 18s rDNA sequencing of marine animals.
• *Genetic diversity (and human equality)*. Simulation of a DNA individuality test.
• *Epigenome*: Comparing the chromatin accessibility of tissue-specific genes by FAIRE.

RESULTS: The results are, in our opinion, very gratifying. The students are enthusiastic, display curiosity and readily master the main aspects of the experimental approach (use of controls, reproducibility, etc.). Those selected for the final talk present the results in a very competent and professional manner, setting an example for the others. As a result, each year more and more students ask and compete to participate to the Eureka project, that is now an essential part of the school's teaching programme, thus confirming the Plutarch's quote: "*The mind is not a vessel to be filled, but a fire to be kindled*".

Topic 1. **Adaptation of microbial sources to an efficient control of the oxidative damage**
Topic 2. **Kinetic mechanism of new inhibitors of the human oncogene Cdc25B**

A. Albino, S. Marco, A. Capasso, A. Bertoni, M.R. Ruocco, E. De Vendittis
Collaborations
R. Rulllo (ISPAAM, CNR); M. Masullo (Università Parthenope di Napoli); A. De Angelis, D. Desiderio, G. Raimo (Università del Molise); A. Lavecchia, F. Sica (Università di Napoli Federico II); A. Di Maro, A. Chambery (Seconda Università di Napoli)

Topic 1. *Streptococcus mutans*, the main responsible of dental caries, and *Streptococcus thermophilus*, a safe microorganism used for preparing dairy products, are facultative anaerobes controlling the levels of ROS through specific antioxidant enzymes. Indeed, both microorganisms possess a 'cambialistic' superoxide dismutase (SOD) for a preventive action against ROS, and an adaptable thioredoxin system for repairing ROS damages. In spite of the great similarities between these microbial sources, great differences exist between *S. mutans* and *S. thermophlus* either in the regulation of SOD functions through the exchange of the metal bound cofactor, or in the reducing power of their thioredoxin system. Nevertheless, both microbial sources have adapted their antioxidant enzymes to an efficient ROS control (1).
Glutathione (GSH) coordinates the antioxidant defence network in aerobic cells. A balance between GSH and its oxidised/nitrosylated forms controls cellular functions, even through post-translational modification of cysteine residues of protein targets. In the psychrophile *Pseudoalteromonas haloplanktis* the role played by GSH is crucial, because of the cold habitat of this source; moreover, GSH causes the S-glutathionylation of various enzymes involved in the oxidative stress control. As in eukarya, GSH biosynthesis occurs through two ATP-dependent reactions, involving distinct activities, γ-glutamyl-cysteine ligase (GshA) and glutathione synthetase (GshB). The detailed characterization of GshB from *P. haloplanktis* was already reported (2); current work concerns an investigation on two putative GshAs from *P. haloplanktis*, in order to reconstitute the enzyme system for GSH biosynthesis in a source highly responsive to glutathione metabolism/homeostasis.
Topic 2. Cdc25B, a phosphatase regulating the cyclin-dependent kinases, represents an attractive drug target for anticancer therapies. New structurally unrelated inhibitors with a different inhibition profile on either human cancer lines as well as on the purifed Cdc25B catalytic domain were identified; a detailed study on their kinetic mechanism of inhibition was carried out (3). Current work is aimed at improving their affinity and specificity, by selecting structural modifications strengthening the therapeutic potential and by evaluating the cytotoxic effect on some cancer cell lines.

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Identification of novel direct targets of microRNAs involved in cellular senescence

Fabiana Passaro, Marika Comegna, Marco Napolitano, Mariangela Succoio, Filiberto Cimino and Raffaella Faraonio

Cellular senescence, a permanent cell cycle arrest triggered by different stimuli, is widely considered a key component of the aging process, as well as a tumor suppressor mechanism. We have recently demonstrated that a set of miRNAs named Senescence-Associated miRs (SAmiRs) is causally related to the induction of cellular senescence in IMR90 cells. In fact, ectopic expression of five SAmiRs in young cells activated a premature senescence program (1). To characterize molecular pathways in which two of them (SAmiR-494 and SAmiR-486-5p) are involved, we searched for direct targets by applying two different strategies.

The first one used 2D-DIGE coupled to mass-spectrometry to profile protein expression induced upon adoptive expression of SAmiR-494. We found that miR-494 significantly down-regulated 121 proteins. Of these, bioinformatic prediction analysis identified 26 potential targets of miR-494, with 7 featured evolutionary conservation of binding sites for miR-494 seed. We validated functional binding sites for hnRNPA3, PDIA3, SYNCRIP/hnRNPQ, and RAD23B. Furthermore, knockdown of hnRNPA3, SYNCRIP and RAD23B demonstrated that hnRNPA3 and, to a lesser extent, RAD23B mirror the phenotype produced by miR-494, blunting cell proliferation and causing upregulation of senescence-associated β-galactosidase activity and DNA damage (2).

In the second strategy, we generated a list of mRNAs down-regulated on senescent human diploid fibroblasts comparing the data of six different microarray gene expression profiles available on PubMed. This comparative analysis allowed us to select 139 mRNAs. Then, we analyzed all 139 mRNAs for the presence of consensus motives for SAmiR-494 and/or for SAmiR-486-5p using four different target prediction algorithms and focusing on the results common to at least two algorithms. This screening allowed us to find 25 putative targets of SAmiR-494 and 11 of SAmiR-486-5p. Validation by luciferase assay led us to identify CDCA2 and ID4 as novel targets of SAmiR-494 and -486-5p, respectively (3).

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Control of cAMP signaling by the Ubiquitin-Proteasome Pathway

Luca Lignitto, Maria Sepe, Monia Porpora, Rossella Delle Donne, Laura Rinaldi, Antonella Arcella, Adriana Gallo, Corrado Garbi, Antonio Feliciello.

Our work impinges on the mechanisms regulating compartmentalized cAMP•PKA signaling by A-Kinase-Anchor-Proteins (AKAPs). AKAPs form local transduction units, which include different signalling/metabolic enzymes, receptors, ion channels, adaptor molecules and mRNAs. Our recent contributions in cAMP signaling have changed our approach to signal-regulated protein degradation **1.** We have demonstrated that ubiquitin-dependent proteolysis of AKAP121 has a relevant role in hypoxia signaling and cell death (ref. 1). In human epithelial cancers (mammary and prostate), upregulation of AKAP121 is linked to metabolic reprogramming of tumor cells. Key mitochondrial targets of AKAP121•PKA action have been identified and currently being characterized. **2.** We have discovered a novel AKAP (praja2) that ubiquitinates and degrades PKA inhibitory (R) subunits. In the brain, degradation of R by the proteasome sustains downstream cAMP signaling (ref. 2). **3.** Recently, we found that praja2 ubiquitylates and degrades Mob1, a core component of NDR/LATS kinase and a positive regulator of the tumour-suppressor Hippo cascade. Degradation of Mob1 through the ubiquitin-proteasome system attenuates the Hippo cascade and sustains glioblastoma growth (ref. 3). Novel praja2 substrates potentially involved in GBM progression have been recently identified. Altogether, our findings suggest the existence of an intimate connection between hypoxia, compartmentalized PKA and the proteasome machinery that may underpin the basic mechanism(s) of cell biology.

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Activation and nuclear translocation of Proline-rich tyrosine kinase2 is induced by stress signals in epithelial cell lines.

MR Carbone, M Labattaglia, E Gionti

Proline-rich tyrosine kinase2 (Pyk2) and Focal adhesion kinase (FAK) are related cytoplasmic tyrosine kinases sharing similar domain organization and nearly 45% sequence identity. FAK is ubiquitously expressed, high level of Pyk2 expression is cell type specific.
Pyk2 is primarily activated in response to a variety of stimuli that increase intracellular Ca+2. FAK has been reported to coordinate adhesion and cytoskeletal dynamics with cell growth and survival. Depending on the cell context Pyk2 has been reported to mediate either cell survival or apoptosis. Subcellular localization is also a critical component of Pyk2/FAK signaling and involves the C-terminal Focal Adhesion Targeting domain and the N-terminal FERM domain showing a nuclear localization signal (NLS).
Nuclear translocation of Pyk2 and FAK has been reported (1,2). Schlaepfer and colleagues showed that Pyk2 (2) enters the nucleus where it can bind p53 through the FERM domain and causes its degradation in a kinase independent manner. Girault and colleagues (3) showed that nuclear accumulation of Pyk2 in neuronal cells is Ca^{+2}/calcineurin-dependent and independent of kinase activity.
Here we show that stress signals induce activation and nuclear translocation of Pyk2 in epithelial cell lines. Activation of Pyk2 is associated with Akt, ERK and p38 MAPK activation.
Stress-induced phosphorylation of Pyk2, Akt and ERK were attenuated by treatment with the inhibitor of ROS (N-acetyl-L-cysteine) and NADPH oxidase (diphenyleneiodonium).
Inhibition of PKC attenuated stress-induced phosphorylation of Pyk2 and potentiates phosphorylation of Akt. Inhibition of Pyk2 had no effect on ERK phosphorylation but potentiated stress-induced phosphorylaion of Akt.. Inhibition of PI 3K also led to enhanced stress-induced phosphorylation of Pyk2.
These data show that NADPH oxidase-derived ROS generation is involved in stress-induced activation of Pyk2, Akt and ERK in epithelial cell lines. Cross-talk between PKC/Pyk2 signaling and PI 3K/Akt signaling may regulate the level of cytoprotection in epithelial cell lines.

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Myc-mediated transcription and Myc-induced DNA damage: two sides of the same coin

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Activated oncogenes generally associate with the induction of DNA damage response (DDR) signaling that induces compensatory tumor-suppressive responses, such as cellular senescence or apoptosis. This phenomenon generally named Oncogene Induced Damage (OID) is exemplified by the c-MYC (henceforth Myc) proto-oncogene. Recent observations have proposed that DNA damage can be associated with Myc overexpression. Indeed, the accumulation of ROS-associated oxidative damage coincided with Myc activation in several cell cultures in vitro. We are currently investigating the molecular mechanisms underlying the Myc capability to induce DDR. Our experimental approaches are based on our recent findings showing that Myc-mediated transcription recruits the histone lysine demethylase LSD1 on its promoter targets. LSD1 is a FAD-containing enzyme, which generates hydrogen peroxide during demethylation with concomitant production of H_2O_2 that determine DNA oxidation. We have demonstrated that accumulation of DNA damage (8-oxodG) co-localizes on Myc targets following Myc-LSD1 recruitment (Amente et al., 2010a, 2010b and 2013). We are currently investigating in a genome-wide approach if DNA oxidation is an intrinsic consequence of Myc-mediated transcription. Using a human mammary Myc-inducible cells (MCF10-MycER), we determine that DDR signaling occurs following Myc activation. To determine if accumulation of DNA damage (8-oxodG) co-localizes on Myc targets, we have performed 8-oxodG immunoprecipitation followed by next generation sequencing analysis (DIPseq). We will present and discuss our genome-wide mapping of Myc-dependent oxidative mark (i.e. 8-oxodG) and the correlation between accumulation of Myc-induced 8-oxodG and Myc binding on its targets.

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The function of the APP/Fe65 complex in the response of the cells to DNA damage: possible implications in neurodegeneration

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The molecular mechanisms underlying neurodegeneration in Alzheimer's disease (AD) are still poorly understood. A key player in the pathogenesis of this disease is the β-Amyloid Precursor Protein (APP). Among the various possible scenarios designed to explain the relationships between APP and AD that of APP loss of function is of particular interest. We are studying the functions of APP-Fe65 complex and the possible consequences of their suppression; in particular, we are focusing on the role of this complex in the nucleus. It is already known that: the interaction of Fe65 with APP results in the "activation" of Fe65 (open conformation); Fe65 is translocated into the nucleus where it interacts with several proteins and with chromatin; both Fe65 and APP suppression are accompanied by an increased sensitivity to DNA damaging agents and by a defective DNA repair; "active" Fe65 interacts with Tau, another known player in the pathogenesis of AD and of other neurodegenerative diseases.
To understand the molecular basis of the involvement of Fe65 in DNA repair our ongoing work is following three main lines. Two of these are aimed at exploring the effects of Fe65 suppression on the DDR and of the possible involvement of Fe65-Tau in these mechanisms. Our data demonstrated that Fe65 null MEFs show alterations of apoptotic response to genotoxic agents and are more susceptible to oncogenic transformation. Interestingly we observed that many human tumors have reduced levels of Fe65. We are now exploring these phenomena in primary neurons.
We have also demonstrated that a fraction of Tau is present in the nucleus, where it interacts with Fe65 and with chromatin. DNA damage results in modified Tau trafficking, post-translational modifications and chromatin recruitment. We are now studying the effects of Tau suppression on DDR. Another line is based on the observation that a ligand of "active" Fe65 is N-WASP, an actin regulatory protein. Many results indicated a role for actin in the nucleus, therefore we recently started to explore whether APP/Fe65 loss of functions could alter actin-based mechanisms in the nucleus.

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Epithelial cell polarity: function and dysfunction

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Polarization of epithelial cells is a complex process directed by external stimuli (such as cell-cell and cell-extracellular matrix adhesion) devoted to the establishment of a unique cytoarchitecture, including a specialized organization of organelles, plasma membrane and cytoskeleton, and to the acquisition of specialized trafficking pathways. In particular, the formation of specialized domains (apical and basolateral) of the plasma membrane displaying different protein and lipid compositions, and the assembly of a full set of junctional complexes, comprising tight and E-cadherin-adherens junctions, is a distinctive feature of epithelial cells. Once polarity is established, this asymmetric distribution is achieved by continuous sorting of newly synthesized components and their regulated internalization. Hence, the integrity of this architecture is crucial for epithelial function. In fact the loss of cell polarity is a hallmark of cancer and other diseases.
The aim of our research group is to identify the molecular machineries required for epithelial polarization in order to understand how cells establish and maintain the polarized phenotype and how these processes are altered in cancer and/or other diseases. By using different models of in vitro cultures we have highlighted the crucial role played by cell matrix and integrin signalling in the epithelial polarization as well as the role of cell-cell interaction (CDH1 and 16) in the thyroid polarization and development. We have also deeply dissected the protein trafficking/sorting in polarized cells, clarifying the role of lipid rafts in protein sorting and demonstrating that protein oligomerization is a key step for the apical sorting of GPI-proteins.
Currently we are investigating these aspects:

1) Role of the GTPase Rac1 in the acquisition and maintenance of polarized phenotype;
2) Characterization of the sorting and trafficking of GPI- and transmembrane proteins to the plasma membrane and identification of the molecular determinants of apical sorting;
3) Analysis of the mechanisms of spatial organization and functional regulation of GPI-proteins;
4) Analysis of epithelial cell dysfunction: role of membrane trafficking in the development of cancer and neurodegenarative diseases.

Identification and characterization of molecules and pathways regulating Pluripotent Stem Cells fate and somatic cell reprogramming.

Parisi S., Battista M., Musto A., Navarra A., Minopoli G., Vocca A. and Russo T.

Pluripotent Stem Cells (PSCs), i.e. Embryonic (ESCs) and induced Pluripotent (iPSCs), represent a powerful and attractive tool for translational medicine. To make this goal feasible, it will be essential to improve our ability to govern the PSC differentiation. In this context, our ongoing projects are aimed at dissecting molecular mechanisms regulating ESC differentiation and somatic cell reprogramming with particular interest in transcription factors (TFs) and miRNAs. We have already identified and characterized a TF (Klf5) that have a crucial role in ESCs (Parisi et al., 2008). We are now trying to decipher the regulatory circuit generated by Klf5 and the TFs Otx2 and Nanog in PSCs and reprogramming (in collaboration with Prof. Simeone). Among the TFs studied, we find of particular interest the function of Hmga2 in the regulation of ESC differentiation and in iPSC generation (in collaboration with Profs Pierantoni and Fusco).
Recently, we have identified a transmembrane protein (Dies1; Aloia et al., 2010) and some miRNAs (Tarantino et al., 2010) all having a crucial role in ESC fate. We have demonstrated the existence of an auto-regulatory loop, involving BMP4, Dies1 and miR-125a, that regulates the proper response of ESCs to the BMP4 stimulus (Parisi et al., 2012). We are now studying the miRNAs that are directly regulated by BMP4 and Nodal/Activin and how these miRNAs modulate ESC differentiation and reprogramming, by balancing these two crucial pathways. We are also searching for compounds that allow to govern PSC differentiation and iPSC induction. In this context, we are studying to role of D-aspartate in ESC differentiation and somatic cell reprogramming (in collaboration with Prof. Usiello).
We have recently developed the procedure to obtain iPSCs from human fibroblasts. In this frame, we have recently generated iPSCs from Down syndrome embryonic fibroblasts to study the molecular mechanisms underlying the defects of the disease (in collaboration with Prof. Nitsch).

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Superoxide dismutase enzymes in cell function and disease

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Reactive oxygen species (ROS) play a key role in pathological and physiological cellular processes. At low concentrations, they regulate many signalling pathways, whereas their overproduction provokes an oxidative stress damaging the cell structures. Cells developed antioxidant defence systems, to protect themselves from ROS damages. An important antioxidant system is represented by the metallo-enzyme superoxide dismutase (SOD), controlling the intracellular and extracellular redox balance through the conversion of superoxide anion into molecular oxygen and hydrogen peroxide. Mammalian tissues contain three forms of SOD: the cytosolic SOD1 (Cu/ZnSOD), the mitochondrial SOD2 (MnSOD), and the extracellular SOD3 (Cu/Zn EcSOD).
The relevance of SOD enzymes in cell function and disease was investigated in various cellular systems: i) neuroblastoma and melanoma cell lines, during diclofenac-induced apoptosis; ii) systemic sclerosis fibroblasts (SScF); iii) ascending aortic aneurysm (AsAA) with tricuspid (TAV) or bicuspid (BAV) aortic valve.
i) The treatment of SH-SY5Y neuroblastoma cell line, as well as of A2058 and SAN melanoma cell lines, with diclofenac, a nonsteroidal anti-inflammatory drug, increased the intracellular ROS levels and activated the mitochondrial pathway of the apoptosis. During the diclofenac-induced apoptosis, an involvement of SOD2 was demonstrated; indeed, both a decrease of protein levels and cytosolic translocation of SOD2 were detected (1,2).
ii) Compared to healthy fibroblasts, dermal SScF showed an increase of both SOD3 gene expression and extracellular enzyme activity (3).
iii) The analysis of protein levels of SOD3, pAkt and Akt revealed a significant reduction of these proteins only in AsAA tissues from BAV patients.
Our studies show the involvement of SOD2 in apoptotic process induced by diclofenac in cancers cells. Furthermore, our results outlined a link between SOD3 and SSc fibroblasts phenotype and propose a possible role of decreased SOD3 protein levels in the pathogenesis of AsAA with BAV, thus confirming that the development of the AsAA proceeds through distinct signalling pathways in TAV or BAV patients.

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Beta-defensin analogs display salt resistant antimicrobial activity and lack toxicity in human epithelial cell lines

Adiponectin is involved in the control of human lung epithelial a549 cell viability

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Human beta-defensins (hBDs) are crucial peptides for the innate immune response and are potential therapeutics against infective diseases. In the last years, we have synthesized 4 analogs of hBD1 and hBD3 wild-type and investigated their potential as antimicrobial drugs evaluating the antimicrobial activities. We have showed that the new analogs have: higher antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis*, even under elevated salt concentrations; Herpes simplex virus type-1 antiviral activity higher than hBD1 and similar to hBD3; and none cytotoxic and genotoxic effects in human epithelial cell lines from lung, colon and pancreas. We also investigated the localization of the new peptides during their incubation with epithelial cells and found that they are distributed on the cell surface from which they are internalized. Finally, we showed that hBD1 and hBD3 are characterized by high resistance to serum degradation. In conclusion, the analogs seem to be promising anti-infective agents.
Adiponectin (Acrp30) is an insulin-sensitizing fat hormone with therapeutic potential for obesity-related diseases. Moreover, it displays anti-inflammatory actions in several tissues. We reported elevated serum levels of Acrp30 in Chronic Obstructive Pulmonary disease patients but the role of the increased levels are not clarified. To this aim, we analyzed the effects of Acrp30 treatment in human lung cells and investigated its role in A549 cells after TNFα treatment. We demonstrated that Acrp30 reduces cell viability; interestingly, in TNFα pre-treated cells, Acrp30 treatment is able to ameliorate the proliferation rate. We found also that the reduction of cell viability by Acrp30 is linked to the inhibition of ERK1/2 while the beneficial effects in co-treatment with cytokines are mediated by activation in ERK1/2 phosphorylation.
Acrp30 could play a central role in the control of local lung inflammatory state, thus Acrp30 may represent a target for the development of new therapeutic approaches.

Food-antioxidant and cancer: a dangerous liaison?

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Gene-environment interaction relates to the additive, synergic, and combinatorial interactions of all the genes of a living organism and all the physical/chemical environmental stimuli that hit the organism, thus, not only controlling health and disease, but, more remarkably, determining the fate of any living organism. Oxidative stress, induced by a wide range of environmental factors is be involved in the development of several human diseases like cancer, atherosclerosis, myocardial infarction and aging. Diet has been recognized as a major environmental determinant of health and the abundance of food antioxidants able to counteract oxidative stress- induced damages is the base for the beneficial effect of Mediterranean and Asian diet. In recent years resveratrol, an antioxidant from grapes, has gained enormous attention because of its potential use in new preventive protective and therapeutic strategies for chronic degenerative diseases including cancer. Resveratrol exerts a wide spectrum of activities able to influence proliferation and signal transduction pathways, immune and inflammatory response. The rationale for the potential therapeutic use of resveratrol against cancer resides on its ability to induce growth arrest, apoptosis, and recently autophagy. However, confusing if not contradictory results due to the strong influence of cellular contest and dose and time of treatment on resveratrol activities and the lack of conclusive clinical trials do not yet allow the safe use of resveratrol in human. To contribute to shade light on this surprising molecule, we are investigating resveratrol effects in response to environmental induced oxidative stress on two model systems of skin and prostate. In particular, we are currently interested in the relation between resveratrol and the oncogene HMGA1, whose over-expression is a common feature of naturally occurring cancers and the tumor suppressor gene HIPK2. Interestingly, both HMGA1 and HIPK2 are influenced by and are able to modulate oxidative stress.

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Non-Canonical Funtions of Carbonic Anhydrase IX in Hypoxic Cells

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Carbonic anhydrase IX (CA IX) is known as a transmembrane protein. It is a well recognized marker of hypoxia and it is involved in pH and survival regulation in hypoxic cells. CA IX is a marker of poor prognosis in many cancer types and it is associated to resistance to chemio- and radio-therapy. So, there is a diffuse interest in inhibiting specifically its function. The main aim of our current activity is to identify molecular and functional interactors of CA IX and, based on the knowledge of its ligands, to contribute to functional characterization of CA IX. Moreover, these interactors may drive design and development of peptide-mimetics interfering with CA IX function. A complex protein network of novel CA IX interactors has been recently highlighted by our group. We found that many of the CA IX protein interactors belong to the family of the ARM and HEAT-repeat containing proteins. Surprisingly, several proteins of the nucleo-cytoplasmatic machinery interacted with CA IX, mostly under hypoxia. These interactions suggested us that CA IX may have a non canonical nuclear functions. Immunofluorescence (IF) analysis demonstrated an accumulation of CA IX in the nuclei and nucleoli of hypoxic cell lines. Putative NLS/NES sequences were identified in the CA IX protein sequence, which were able to affect distribution of the reporter protein GFP inside the cell. Collectively, our data suggest that the subcellular localization and the functions of CA IX are more complex than previously thought. CA IX may have intracellular functions different from those already known at the plasma membrane. Indeed, we found CA IX on transcribed chromatin. Our data may shed light on unsuspected features in CA IX biology and its role in molecular mechanisms of cancer.

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