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PRESENTAZIONI ORALI

Genome wide mapping of the MBF-1 binding sites during embryogenesis of the sea urchin reveals it is a chromatin organizer

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The Zinc-finger MBF1 factor is a transcription activator involved in the expression of the early histone genes during development of the sea urchin embryo (1). The DNA-binding domain of MBF1 shares high sequence similarity with that of the CTCF chromatin organizer but, unexpectedly, extensive *in silico* analysis failed to identify the sea urchin CTCF ortholog (2, 3). This led us to speculate that MBF1 could have co-opted the function of CTCF during evolution of the echinoderms. To support this hypothesis, we performed the genome-wide MBF1-binding sites mapping in the *P. lividus* genome, by chromatin immunoprecipitation coupled to next generation sequencing (ChIP-Seq). We observed that MBF1 binding motifs are spread across the genome, with a CCCTC core sequence showing perfect conservation with the mammalian CTCF binding element. In particular, MBF1 binds to the promoter regions of hundreds of target genes. Among others, we confirmed the specific interaction with the promoters of histone and *Hox* genes, and observed the full evolutionary conservation of these binding sites in *P. lividus* and *S. purpuratus* species. Next, to appraise globally the functional meaning of binding events we analyzed the MBF1 occupancy in chromatin samples derived from embryos exposed to compounds, such as Lithium and Zinc, that impair axial pattering. Comparison with controls revealed differential MBF1 recruitment on selected genes reflecting differentially regulated mechanisms in treated embryos. The molecular pathways impacted by Li and Zn include cell signaling, gene transcription, DNA repair, and chromatin condensation. Collectively, our observations highlight the DNA binding potency of MBF1, strongly suggesting that it could act both as a transcription factor of its target genes and a general chromatin organizer.

[1] Cavalieri, V et al. (2009) *Nucleic Acid Res*, **37**,7407-15.
[2] Heger , P et al. (2012) *PNAS*, **109**, 17507-12.
[3] Cavalieri, V et al. (2013) *Plos Genetics*, **9**, e1003847.

NiII and ZnII Schiff base complexes: B-DNA vs. G4-DNA binding

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Last decades discoveries about implications of DNA polymorphism in cancerous events have turned on the interest of researchers about targeting non-canonical DNA structures. Among them G-quadruplexes (G4), arising from guanine-rich regions of DNA in which tetrads of guanines are linked by Hoogsteen hydrogen bonds, have been found to naturally occur in human telomeric regions where they inhibit reverse transcriptase activity of telomerase.

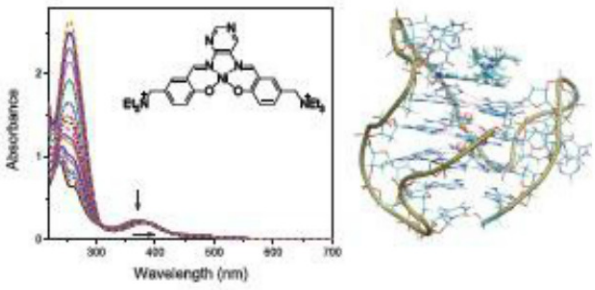

Figure 1. UV-Vis spectra of the reported NiII complex in presence of increasing amounts of telomeric quadruplex (left); a representative snapshot taken from the molecular dynamics simulation showing the interaction between between NiII complex and telomeric quadruplex (right).

This enzyme is overexpressed in 90% of tumours where it turns on to elongate telomeres, granting unlimited proliferation capability to the cancerous cells. For such a reason,stabilizing G4s in telomeres might become a powerful strategy to contrast abnormal telomerase activity thus arresting cancer cells proliferation. We have recently reported on the G4-DNA interaction ability of a series of Salnaphen-like metal complexes. Indeed, Schiff base metal complexes, derived from N,N'-bridged tetradentate ligands, involving N2O2 donor atoms, present very favourable features to act as G4 binders. Therefore NiII and ZnII Salpyrim-like complexes have been synthesised and their interaction towards B-DNA and telomeric G-quadruplex have been tested by means of spectrophotometric, hydrodynamic and computational approaches.
The data collectively suggest that only NiII complex is capable of strong interactions both with B- both with G4-DNA: it acts as a typical B-DNA intercalator but it also binds to G-quadruplexes by direct end-stacking, stabilizing the oligonucleotide secondary structure. Remarkably, it has been found a 10 fold higher affinity constant towards telomeric quadruplex-DNA than that towards B-DNA, highlighting a selective interaction of the NiII complex. Experiments to evaluate the biological activity of the two complexes against cancer cell lines are currently ongoing.

Isolation and characterization of sea urchin *P. lividus* microbiota from coelomic fluid

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The study of the microbiota is a subject of considerable and growing interest since it is drawing new important perspectives in the life sciences concerning the functional relationships between metazoans and microbial cells. In fact, it has already shown that the endogenous microbial community affects various physiological activities of multicellular organisms. The coelomic cavity of echinoderms contains a fluid in which coelomocytes are reported to exert immune functions like phagocytosis, opsonization and production of antimicrobial agents against marine bacteria [1, 2]. However, up to day nothing is known about the endogenous bacterial population of coelomic fluid. We focused on this issue, and, to this aim, both bacterial culture-dependent and –independent approaches were adopted. By the former approach, we isolated 8 distinct Gram-negative marine bacterial strains identified for their 16S rDNA sequence. Interestingly, almost all isolated strains show a considerable extracellular hydrolytic activity. Moreover, one of them exerts antimicrobial effect against Gram-negative bacteria, including most of the other strains isolated from the coelomic fluid. Finally, molecular investigation on metagenomic DNA composition is currently ongoing using Next Generation Sequence Technology. This study not only suggests insights on functional interaction between sea urchin and marine microorganisms, but also could provide a novel source of biochemical diversity for the production of bioactive compounds and enzymes that can find biotechnological applications.
[1] Remziye Deveci et al. (2015). *Journal of Morphology* ***276(5)****:583-8*
[2] Stabili L et al. (1996). *Comp Biochem Physiol B Biochem Mol Biol* ***113(3)****:639-44*

DNA cytosine methylation modulates *Streptomyces coelicolor* morphological and physiological differentiation

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The role of DNA cytosine methylation in prokaryotes has not been deeply investigated. In *Escherichia coli* cytosine methylation regulates gene expression during the stationary phase and cytosine hypermethylation

leads to chromosomal DNA cleavage and cell death. *Streptomyces coelicolor* is a mycelial soil microorganism, producer of several antibiotics, with a complex life cycle that includes three different cell types: unigenomic spores, a compartmentalized mycelium (MI) and a multinucleated mycelium (MII).
The main objectives of this study were to analyze the DNA cytosine methylome along the growth of *Streptomyces coelicolor* and to investigate the relationship between DNA cytosine methylation and morphological/physiological differentiation. Methylome analysis revealed that the global level of methylated cytosines, detected in the region comprised between -400 and +100 bp of genes, changes along the growth during development both on solid GYM and in liquid R5A media. Bioinformatic analysis of DNA cytosine methylome, showed three cytosine methylation motifs, GGC^{m}CGG, GCC^{m}CG and C^{m}GGGC, in the upstream region of genes involved in morphological and physiological differentiation. Methylome results were compared to transcriptomic analysis, previously carried out, showing a correlation between the cytosine methylation motifs and gene expression. In addition, liquid and solid cultures were treated with 5-aza-2'-deoxycytidine (aza-dC, a cytidine analogous that inhibits DNA-methyltransferase activity) demonstrating that methylation influences *S. coelicolor* germination and antibiotic production both in liquid and on solid culture and sporulation on solid culture. Altogether these results demonstrate a strong relationship between DNA cytosine methylation and morphological/physiological differentiation.

*Characterization of immunoregulatory mechanisms induced by **Parietaria judaica** Par j 1 allergen*

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Parietaria judaica (Pj) pollen is one of the major source of allergens in all the countries around the Mediterranean area (Colombo et al. 2003). The composition of the allergenic extracts of the Pj pollen has been studied in details showing that its major allergens (Parj1 and Parj2) are components of the LTP family (Colombo et al. 2003; Amoresano et al. 2003). During a tolerogenic immune response in healthy subjects, the major allergen of the Parietaria pollen Parj1 is capable of inducing both the arms of the innate and adaptive human immune system, through the selection of IFN-γ^{+} and IL-10^{+}NK cells characterized by a CD56^{bright}CD16^{dim}CD335^{+} immunoregulatory phenotype, and a CD4^{+}CD25^{+} FOXP3^{+}IL10^{-} T cells population (pTregs) (Bonura et al. 2013). In this respect, published papers demonstrated that Tregs modulate NK cells function through cell-cell conctact mechanism mediated by the membrane TGF-β (Ghiringhelli et al. 2005), associated in a complex with LAP (Latency-Associated Peptide) and GARP (Glycoprotein A Repetitions Predominant - LRRC32). In order to investigate the interaction between these two cell populations, we look at the expression of some phenotypic and functional markers by Multiparametric Flow Cytometry. After seven days of stimulation with Parj1 we observed: 1) a decrease in the proliferation of CD56^{bright}CD16^{dim} NK population and the modulation of the activatory and inibitory receptors, such as NKG2D (CD314) and NKG2A (CD159a); 2) the modulation of LAP, GARP and other surface markers, such as CD39, on the CD4^{+}CD25^{+} Treg population. In conclusion we are on the way to delineate the mechanisms behind the development of peripheral tolerance to allergens. The kwoledge of these events may represent a key point for the progress of new therapeutic strategies for the cure of allergic disorders.

Fine characterization of immunological mechanisms mediated by the major allergens of *Parietaria judaica* and by a hypoallergenic hybrid, rPjEDcys

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Allergy is a hypersensitivity disease IgE-mediated, affecting more than 25% of the population. Actually the only curative treatment of allergies is Allergen-Specific Immunotherapy (SIT). Recombinant hypoallergenic allergen derivatives with reduced allergenic activity have been engineered to reduce side effects during SIT. *Parietaria judaica* (P*j*) pollen contains two major allergens, Par j 1 and expressing disulphide bond variants of Par j 1 and Par j 2, was generated. The aim of this research project is to compare the immunological mechanisms activated by the major allergens of *Pj* and by rPjEDcys. *In vitro* analysis suggested that rPjEDcys has a reduced allergenity and maintains T cells reactivity. In particular we showed that PBMC of P*j* allergic patients stimulated *in vitro* with the hybrid and the *wild-type* recombinant allergens scored a percentage of proliferating $CD4^+$ and $CD56^+$ cells higher than unstimulated samples. Furthermore, cytokine secretion assays on $CD4^+$ cells demonstrated that rPjEDcys reduces the secretion of two Th2 cytokines that are critical in the development of allergy such as IL-5 and IL-13. Furthermore we observed the selection of a putative pTreg cell subset (defined as $CD4^+$ $CD25^{++}$ $CD127^-$) in both the w.t. mixture and the rPjEDcys. We characterized these cells at molecular level by REAL-TIME PCR. Moreover, we addressed the kinetic of functional surface marker expression, such as GARP (Glycoprotein A Repetitions Predominant), LAP (Latency-Associated Peptide) and CD39 on $CD4^+$ cells. Our analyses demonstrated that rPjEDcys induces a number of GARP-LAP-CD39 co-expressing cells higher than *wild-type* recombinant allergens. These results suggest that rPjEDcys represents a useful approach for immunotherapy of allergic disease.

Cigarette smoke extract promotes Acetylcholine mediated inflammation and oxidative stress by PEBP1/Raf-mediated MEK and ERK pathway in human bronchial epithelial cells

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Acetylcholine (ACh) promotes oxidative/nitrosative stress in bronchial epithelial cells of COPD patients. The use of anticholinergic and long-acting β2 agonists in the treatment of COPD maximize bronchodilatation and regulate oxidative/nitrosative stress in bronchial epithelial cells. Cigarette smoke extracts (CSE) and Acetylcholine generate the increased expression and activation of m3 muscarinic receptors (mAChR M3), through PEBP1 dissociation. Tiotropium decrease the proinflammatory activity of Acetylcholine and reduces the activity of ACh in guinea pig model of neutrophilic inflammation and remodeling in COPD. β2-Adrenergic receptors are coupled to Gs, where stimulation by a β-agonist activates adenylate cyclase and increases 3,5_adenosine monophosphate (cAMP) level. We aimed to investigate whether the long-term exposure to CSE (0 to 20% for 7 days) promotes inflammation and oxidative/nitrosative stress production in bronchial epithelial cell line (16HBE), via PEBP1 Raf-mediated MEK and ERK pathway activation, by autocrine Acetylcholine. We evaluated the ACh expression and Ros production by flowcytometry, ChAT, M3, NOX4, p-β2AR, PEBP1and ERk1/2 phosphorylation by western blot analysis; the IL-8 release and cAMP levels by ELISA. The 16HBE were preteated with CSE for long-term exposure and Tiotropium and Olodaterol with and without The Hemicholinium (HCh), a potent choline uptake blocker. We showed increased levels of p-β2AR, pPEBP1, M3, ChAT, ACh expression, pERK1/2, Ros, IL-8 and NOX4 in CSE treated 16HBE for long-term exposure compared to untreated cells. HCh reducing levels of ACh synthesis

and binding reduced PEBP1 and ERK1/2 phosphorylation as well as Ros, Nox4 and IL-8 production in CSE treated cells. Tiotropium or Olodaterol reduce the levels of Ros, NoX4, IL-8 and ACh expression. CSE pre-treatment decrease OLO-induced cAMP release to a greater degree than prior TIO treatment. Stimulation of cells with TIO and OLO in combination for 10 minutes induced a high level of cAMP release.

Cigarette smoke impairs Sirt1 activity and promotes pro-inflammatory responses in bronchial epithelial cells

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Cigarette smoke is the major risk factor for chronic obstructive pulmonary disease (COPD), a disease where inflammation and aging are intertwined. Sirtuin (Sirt)1 is an anti-ageing factors that removes acetyl moieties and activates FoxO3, a transcriptional factor which controls cell cycle progression, cell death and inflammation and protects the cell from oxidative stress. In the present study we investigated the relationship between these anti-aging factors and inflammatory processes (NF-κB, IL-8 and CCL20 expression) in response to cigarette smoke. IL-8 and CCL20 were selected as markers for innate and adaptive responses. 16HBE cells and primary bronchial epithelial cells isolated from COPD patients and healthy controls, pre-treated with/without the Sirt1 inhibitor, Sirtinol, were stimulated with increasing concentrations of cigarette smoke extracts (CSE). The nuclear accumulation of Sirt1, FoxO3 and NF-κB, deacetylase activity of Sirt1 and IL-8 and CCL20 expression (protein and mRNA) were evaluated. The obtained results showed that (i) CSE decreases the activity and nuclear levels of Sirt1 in 16HBE cells; (ii) CSE reduces FoxO3 in 16HBE and in primary bronchial epithelial cells from healthy subjects; (iii) the constitutive expression of FoxO3 was more down-regulated in primary bronchial epithelial cells from COPD subjects than from healthy controls; (iv) CSE increased NF-κB and IL-8 expression and decreased CCL20 expression. Pretreatment with Sirtinol reduces FoxO3 and increased NF-κB and IL-8 expression but it had no effect on CCL20 expression. These data suggest that cigarette smoke impairs the function of Sirt1 leading to deregulation of FoxO3 and NF-κB activity, modifying the cellular ageing and inflammatory processes with a prevalent activity of innate immune responses.

Inflammatory reaction and isolation of multifunctional bioactive molecules in cnidarians: from Immunobiology to Blue Biotechnology

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The phylum of Cnidaria is one of the first branches in the tree of animal life to provide crucial insights on the evolution of immunity. Cnidarians are diblastic aquatic animals with radial symmetry and they are the simplest multicellular organisms that have reached the level of tissue organization. The renewed interest in the study of immunity in Cnidaria has led to additional information to the scenario of the first stages of immunity evolution revealing the cellular processes involved in symbiosis, in the regulation of homeostasis and in the fight against infections. We investigated the inflammatory response in Cnidarian following injection of various substances different in type and dimension, and observed clear, strong and specific reactions especially after injection of bacteria. The enzymes evaluation (protease, phosphatase and esterase), showing how the injection of different bacterial strains alters the expression of these enzymes suggesting a correlation between the appearance of the inflammatory reaction and the modification of enzymatic

activities. The Cnidaria phylum has evolved using biotoxins as defense or predation mechanisms for ensure survival in hostile and competitive environments such as the seas and oceans indeed the tissues and the mucus produced by cnidarians are involved in immune defense and contain a large variety of toxins such enzymes, potent pore forming toxins, and neurotoxins. They could also take advantage of the multi-functionality of some of their toxins. The bioactive molecules were characterized and purified by biological assays, acid extraction, HPLC purifications, mass spectroscopy and peptide synthesis. Here, we show the cnidarian bioactive molecules as antimicrobial peptides and enzymes in order to draw important applications in fields ranging from pharmacology to cultural heritage.

Characterization of immunomodulatory activities of Ci8short peptide induced by LPS from Ciona intestinalis

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The dysregulation of the immune response plays an important role in many diseases. Therefore the study of different cell types with immunomodulatory activities is critical for innovative therapeutic approaches. We recently isolated (Vizzini et al. 2013) a LPS-induced peptide (Ci8short) from the ascidian *Ciona intestinalis.* This peptide shows a peculiar amino acids composition suggesting the possibility that it can act as an Host Defense Peptide. For this reason, the immunological properties of the Ci8short peptide were studied by using human PBMC from healthy subjects *in vitro.* As first result, we were able to demonstrate that this peptide did not show cytotoxic or/and hemophilic activities in *vitro*. Furthermore, we observed that the Ci8short displays some immune activities showing the ability to preferentially induce the proliferation of human $CD4^+$ cells at 7 days. Following this line of evidence, we decided to perform a time course looking at the appearance of $CD4^+/CD25^+$ cells after Ci8short stimulation demonstrating that this peptide was able to select peptide-specific effector cells. In particular we demonstrated that Ci8short induces the secretion of IFN-γ, IL-10 and IL-17 cytokines by human $CD4^+$ cells. To further characterize $CD4^+/CD25^+$ cells, we evaluated the expression of an immunophenotypical marker such as the CD127 and the presence of the GARP (Glycoprotein A Repetitions Predominant), LAP (Latency-Associated Peptide) and CD39 functional markers. From this analysis, we showed that the Ci8short peptide induces the selection of $CD4^+/GARP^+/LAP^+/CD39^+$ at 7 days. The results obtained in this study will be useful for the understanding the Ci8short peptide mediated immunological mechanisms as it may have potential to be developed as a novel immune regulatory adjuvant.

Isolation of a novel LPS-induced component of the ML superfamily in *Ciona intestinalis*

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The ML (MD-2-related Lipid-recognition) superfamily contains a large set of genes encoding proteins such as MD-1, MD-2, NiemannPick type C2 (NPC2) protein, the GM2 activator protein and the mite allergen Der p 2. Members of the ML domain play important role in lipid metabolism (sterol homeostasis and steroid biosynthesis) but also in innate immune signal pathways (LPS induced signalling). In invertebrates, a few ML genes have been identified in Drosophila melanogaster (Shi et al., 2012), in the shrimp Litopenaeus

vannamei (Liao et al., 2011) and in the Camponotus japonicus (Ishida et al., 2014). In this study, we report the identification of the first component of the ML superfamily in the invertebrate *Ciona intestinalis* by means of a subtractive hybridization strategy. Sequence homology and phylogenetic analysis showed that this protein forms a specific clade with vertebrate components of the Niemann-Pick type C2 protein and, for this reason, it has been named Ci-NPC2. The putative Ci-NPC2 is a 150 amino acids long protein with a short signal peptide, seven cysteine residues, three putative lipid binding site and a three-dimensional model showing a characteristic b-strand structure. Gene expression analysis demonstrated that the Ci-NPC2 protein is positively upregulated after LPS inoculum with a peak of expression 1 h after challenge. Finally, *in-situ* hybridization demonstrated that the Ci-NPC2 protein is preferentially expressed in hemocytes inside the vessel lumen.

Janus-Faced role of microRNA let-7d in osteosarcoma 3AB-OS cancer stem cells

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Osteosarcoma (OS) is the most common malignancy of bone in children and adolescents. It is a highly invasive and metastatic bone-malignancy because of which, despite therapeutic advances, 30%-50% of patients still die of pulmonary metastasis. As a consequence, there is an urgent need to identify new therapeutic strategies to improve the clinical outcome of the patients. Advances in OS treatment are inconceivable without better understanding of molecular mechanisms of osteosarcomagenesis and, especially, metastatic processes. Growing evidence suggests that cancer stem cells (CSCs), which have self-renewing and malignant potential, are at the root of tumor growth and relapse. Thus, a challenge for innovative therapy is their identification and eradication. Here, we have used the 3AB-OS CSCs, a cell line previously produced in our laboratory from the OS-MG63 cells, which was genetically, molecularly and functionally characterized. This study was focused on the role of let-7d miRNA –previously found by us to be downregulated in 3AB-OS-CSCs- in managing their stemness properties. We have found that let-7d-overexpression reduces cell proliferation by both decreasing CCND2 and E2F2 cell-cycle-activators and increasing p21 and p27 CDK-inhibitors. Let-7d also reduces sarcosphere- and colony-forming ability and the expression of Oct3/4, Sox2, Nanog, Lin28B and HMGA2, key regulators of cancer cell stemness. Moreover, let-7d induces mesenchymal-to-epithelium-transition, as shown by both N-Cadherin-E-cadherin-switch and vimentin decrease. Surprisingly, this switch was accompanied by enhanced migratory/invasive capacities and by increases in MMP9, CXCR4 and VersicanV1. Let-7d also reduced the resistance to serum starvation and chemotherapy. A decrease in caspase-3 with an increase in Bcl-2 was also observed. Overall, this study shows that let-7d -displaying both suppressor and oncogenic functions- behaves as a Janus-Faced miRNA. Thus, we suggest that, before prospecting new therapeutic strategies by let-7d modulation, it is urgent to better understand its functions.

Plasmatic microRNAs profiles in subjects with clinical manifestations referable to Fabry Disease

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Fabry disease (FD) is an X-linked lysosomal enzymopathy caused by mutations in the GLA gene coding for alpha-galactosidase A. Even though the knowledge of the disease has improved in the last few years FD is still difficult to diagnose. Subjects showing clinical manifestations referable to FD can be classified in 4 categories:1) patients with the classical form of the disease and exonic mutations in the GLA gene, 2) patients with the atypical variants of the disease and exonic mutations in the GLA gene, 3) symptomatic subjects with intronic mutations of the GLA gene, 4) symptomatic subjects without mutations of the GLA gene. In the first two categories the presence of mutations in the GLA gene is sufficient to confirm clinical diagnosis. Regarding the other two categories of subjects symptomatics, without exonic mutations in the GLA gene, whom represent the 98% of patients, new diagnostic tools are required. The aim of this study was to evaluate the microRNAs profiles as diagnostic tool in subjects in whom the genetic analysis does not confirm the clinical diagnosis of FD. We performed a pilot experiment by profiling 800 microRNA in plasma of patients with clinical manifestations referable to FD and control subjects, in order to identify microRNAs related to the disease and, eventually, microRNAs specific for the 4 categories of subjects described before. To date, we identified a 11 microRNAs profile that separates the subjects in two main categories: subjects with exonic mutation and subjects without exonic nutation. These results suggest a correlation between the microRNAs profile and the pathogenetic mechanism. Moreover, the gene-ontology charecterization of these 11 microRNAs suggests the involvement of autophagy process in FD. In conclusion, these preliminary results showed that plasmatic microRNAs could be used as diagnostic markers and that their presence in plasma could be linked to pathogenic mechanisms responsible for FD.

A sample-specific miRNA target prediction algorithm

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MicroRNAs (miRNAs) are small non-coding RNA molecules mediating the translational regulation of target mRNAs. Mature miRNAs are used as a template by the RNA-induced silencing complex (RISC) to recognize the complementary mRNAs that have to be regulated. Up to 60% of human genes are putative targets of one or more miRNAs. Several prediction tools are available to suggest putative miRNA targets, however, none of these tools takes into account the multifaceted network of miRNA-mRNA interactions, which involves competition and collaboration effects that are crucial in miRNA regulation. One efficient way to experimentally obtain the complete picture of which mRNAs are targeted by the RISC complex is to analyze the expression profile of samples obtained by the immunoprecipitation (IP) of RISC proteins. The results are contingent on the specific miRNA expression profile of the input sample, however, no specific information is provided about which miRNA targets which mRNA. We aim to model the miRNA-mRNA interaction network by integrating miRNA-target computationally predicted interactions with the miRNAs and mRNAs expression profiles, with the final goal of obtaining sample-specific miRNA target prediction results. We used the breast cancer MCF-7 cells as out test bed, analyzing samples derived from the IP of two RISC proteins, AGO2 and GW182, and correspondent input and flow-through, by microarray (Agilent). By combining the experimentally produced expression profiles and the computationally predicted binding site

information, we selected the most relevant features able to efficiently predict the targets overexpressed in the IP samples. Finally, we trained a support vector regression model to predict the outcome of an AGO2 or GW182 IP experiment, by simply using as input the information about the input sample. In the next future, we will use our model to predict the IP expression profile obtained from perturbed MCF-7 cells, e.g. after the silencing of specific miRNAs.

Role of protein lipidation in exosome biogenesis and cargo selection

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Protein S-palmitoylation is a reversible post-translational modification (PTM) that regulates many key biological processes, such as protein localization to membranes or to confined membrane microdomains, protein interaction and protein conformation/stability; however the full extent and functions of protein Spalmitoylation remain widely unexplored. It has been recently reported that different lipidations (*i.e* palmitoylation, myristoylation) are able to target a highly oligomeric recombinant cytoplasmic protein into secreted vesicles, pointing also to an essential role of palmitoylation in extracellular vesicles (EVs) cargo selection. In this context is intriguing to observe that proteins more often identified in EVs of diverse sources, such as tetraspanins, heat shock proteins and adaptor proteins, are palmitoylated or bear the predictive site of palmitoylation. Alix is one of the most abundant protein in EVs (*i.e.* exosome) and is involved in many molecular processes, including EV biogenesis. We have previously demonstrated that skeletal muscle (SkM) cells can release Alix-positive EVs, revealing the importance of exosomes in SkM biology and suggesting a new paradigm for understanding how muscles communicate with other organs, such as adipose tissue, bones, the brain or tumors. In silico and biochemical analyses allowed us to determine that Alix is palmitoylated and that palmitoylation inhibition, through a specific inhibitor (2-Br-Palmitate, 2BP), altered its structure and subcellular localization. We also proved that the inhibition of palmitoylation influences the number, size and heterogeneity of SkM-derived EVs as well as the distribution of exosomespecific biomarkers. According to our results, palmitoylation might regulate the proper function of Alix in SkM EV biogenesis, support the interactions among the exosome-specific regulators/biomarkers and maintain proper EV membrane structural organization. We propose that protein palmitoylation might represent a key PTM for the selective biogenesis of EV sub-pool and/or for the loading of specific EV cargo. A better understanding of EV biogenesis and function would pave a way for a possible application of SkMderived exosomes as a novel cell-based therapy for muscle degenerative diseases.

Effect of mesoangioblast extracellular vesicle on cell migration and vessel formation of human endothelial cells

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The discovery that extracellular vesicles (EV) represent an important mediator of cell-to-cell communication has added a novel understanding to regenerative medicine. We investigated on the ability of isolated mouse mesoangioblast EV to have an effct on human endothelial cells (ECV304) to: **1.** modify the migration capability and **2.** induce the differentiation versus *capillary-like structures.* We first established that EV were able to be internalize into ECV304 after 24 h of incubation realising their content into cells. We first verified whether EV were able to modify positively the human ECV304 migration ability by wound healing assays. We have demonstrated that the addition of mesoangioblast EV to the growth medium of ECV304 increased

their migration capability in a dose dependent manner. Moreover, we found out that ECV304, incubated on growth factor reduced matrigel, modulate either positively or negatively their ability to form *capillary-like structures* depending on different concentrations of EV addition, and the use of neutralizing antibodies against FGF-2 and VEGF suggests that they are not the only factors responsible for *capillary-like structures* formation. Furthermore, the transcriptome analysis of mesoangioblast EV showed six overrepresented transcripts related to angiogenesis process and at least other six transcripts were involved in actin cytoskeleton remodulation and cell migration. These findings let us to hypothesize that EV, might be used to change the destiny of other cells.

Correlation between obesity, Irisin and glycemia

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Obesity is a multifactorial disease that involves a series of metabolic dysregulation and the possibility of differentiating adipose stem cells from white fat to brown fat to induce a thermogenic activation and increased energy expenditure which is currently considered as a major promising approach to combat obesity and its complications. A new miochina “irisina” which decrypts active metabolic white fat and turns it into brown fat. This molecule was originally developed because it was released in the course of physical activity. The Irisina (112 amino acids) is a protein fibronectin type III that was out of the proteolytic cleavage of the FNDC5 protein (Fibronectin Domain-Containing Protein 5) which seems to respond positively to exercise. The evaluation of this miochina that seems not to correlate with the nutritional regime is of extreme importance as to date there are few analysis systems sensitive enough to detect it. The purpose of our study was to examine a cohort of individuals (age range 30-70) in good health by relating the main parameters of blood count, clinical chemistry and oxidative stress with the amount of circulating Irisina. Subjects were grouped by age, gender and physical activity. The first results showed consistent correlation between the increase in oxidative stress with age and a lower concentration of Irisina. In normoglycemic subjects we found an interesting correlation with blood glucose levels and an increase in oxidative stress levels.

Serpinopathies: From molecular studies to therapeutic interventions

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Serpinopathies are a class of genetic diseases related to the deficiency of a serpin (SERin Protease Inhibitor) and/or its accumulation as polymer chain in the cell of synthesis. In our studies, we deal with (i) the best-known α_1-antitrypsin deficiency, caused by mutations in α_1-antitrypsin (AAT) determining polymer accumulation in the hepatocytes and lack of inhibition of lung proteases; (ii) the Hereditary Angio-Edema, caused by a poor activity of mutated C1-Inhibitor; (iii) the Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB), related to the accumulation of neuroserpin (NS) polymers within neuron endoplasmic reticulum. We use different biophysical techniques (such as Optical Spectroscopies, Dynamic Light Scattering, Chromatography, Fluorescence Correlation Spectroscopy, Atomic Force Spectroscopy, Molecular Dynamics) to address the structure and function of serpin conformational states. In particular, in order to approach the cellular environment, we focused both on the effects of a crowded environment on serpin function and diffusion and on the role of glycosylation. We have shown how N-linked glycosylation relates to the stability and polymerization of AAT in vitro, as well as to intracellular misfolding, degradation and polymerization of NS. We have also used small angle X-ray scattering (SAXS) to obtain a low-resolution structure of AAT and NS polymers, which sheds light on the ongoing debate on serpin polymer

structure. Such structural achievements are used as a starting point for a pharmacological treatment of serpinopathies, which remains an unmet challenge. We started by performing a large in silico screening of small molecules by molecular docking. Further, we recently identified a small natural compound able to prevent NS polymerization, by specific binding.

Targeting TACE in neuroinflammation and inflammatory arthritis

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Proteolytic processing and release of cell-anchored protein, the so called ectodomain shedding, is a post-translational modification that regulates several biological processes. A number of cytokines and growth factors, including TNFa, need to undergo proteolytic shedding in order to be activated. Shedding of cell-receptors can dampen specific signaling pathways, and shed receptors can sequester soluble ligands, thereby regulating cell responses. The major class of proteases involved in ectodomain shedding is the "disintegrin and metalloproteinases" (ADAMs). A member of this family, ADAM17 (also known as TNFa-convertase or TACE), plays a crucial role in inflammation for its ability to release soluble TNFa, thus triggering pro-inflammatory stimuli. The activity of TACE must be finely tuned. Indeed, its dysregulated activity is associated with several inflammatory disorders, including rheumatoid arthritis (RA), and release of TNFa has been linked with neuroinflammation and neuronal loss in a number of neurodegenerative diseases, including Alzheimer's disease (AD). Herein, we show how two physiological regulatory mechanisms of TACE can be targeted in order to develop therapies that may be beneficial in AD and RA.
- *iRhom2/TACE in AD*. iRhom2 ("inactive-rhomboid-2) has recently emerged as a key regulator of TACE, as it guides its maturation through the ER-Golgi secretory pathway, specifically in immune cells. Genetic ablation of iRhom2 leads to lack of mature TACE and subsequent TNFa release in macrophages/microglia. We have crossed an iRhom2$^{-/-}$ mouse with an AD mouse model (APP/PS1) in order to study the role of iRhom2 in AD. Furthermore, we have investigated the substrate repertoire of iRhom2/TACE in macrophages/microglia by proteomics.
- *T3TRAP in RA*. TIMP-3, the endogenous inhibitor of TACE, is a secreted protein, whose levels are regulated by endocytosis mediated by the low-density-lipoprotein-receptor-related protein-1. We developed a molecule that traps TIMP-3 extracellularly, thereby inhibiting TACE and other metalloproteinases involved in RA.

Identification of p65(-1), a new isoform of the NF-kB complex, by 2D-electrophoresis samples from liver tissue

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Nuclear Factor kB (NF-kB) consist of a family of transcription factors that play critical roles in inflammation, immunity, cell proliferation, differentiation, and survival. As a result, deregulation of NF-kB activity is linked to autoimmune and metabolic diseases, inflammatory disorders and cancer. In mammals there are five members of transcription factor NF-kB: *Rel*A (p65), *Rel*B, c-*Rel*, p50 and p52. All the members of the family share a conserved long amino-terminal *"Rel Homology Domain"* (RHD) necessary for: DNA binding, dimerization, interaction with inhibitors (IkB) and nuclear translocation. Under resting conditions, the dimer p65/p50 is bound to inhibitory IkB proteins,

which sequester NF-kB complex in the cytoplasm. After a specific signal, NF-kB is released from IkB and translocates to the nucleus to control gene expression. The constitutive activation of NF-kB has been observed in many human cancers such as hepatocellular carcinoma. In some types of cancer, NF-kB activation is supposed to be the result of an inflammatory increase or as a consequence of an inflammatory microenvironment during the maturation process of the malignant tumor. The loss of NF-kB inducibility leads to a deregulation of the expression of genes involved in cell cycle regulation, apoptosis, migration and cell adhesion, then it is evident a hold relationship between NF-kB and carcinogenesis. In mouse and in human it has been described a new isoform of p65, called p65(-1). This new isoform contains an unknown exon (exon -1) located upstream to the first known exon of *Rel*A, codifing for p65 (exon 0). We identify the expression of p65(-1) by 2D-electrophoresis, of protein samples from liver tissue belonging to patients affected and not by liver diseases. The results obtained show a different pattern expression in liver samples with cirrhosis and hepatocellular carcinoma.

New proteomic evidence on decorin effects on breast cancer cells

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The establishment of a dynamic crosstalk between the malignant cells and several components of the extracellular matrix (ECM) is a crucial step of the tumor progression. The ECM is a highly intricate microenvironment in which many signals exert opposite effects on the tumor cells. Historically our research group focused the attention on the molecular mechanisms underlying the effects of ECM molecules on the behavior of breast cancer cells in vitro. In this context, the decorin, a small leucine-rich proteoglycan (SLRP) involved in the collagen fibrillogenesis, was found to play an "anti-oncogenic" reaction by affecting the growth and motility in vitro of cancer cells. The aim of the present study was to improve the knowledge about the effect of ectopic decorin on the breast cancer cells, starting from the results previously reported by Pucci-Minafra et al. 2008 (Connect Tissue Res. doi: 10.1080/03008200701820443), in collaboration with the universities of Pavia and Bologna. The experimental model used for this purpose was represented by the 8701-BC breast cancer cell line and by its clone, called DEC-C2, obtained by transfection of 8701-BC cells for the synthesis and secretion of ectopic decorin. The entire protein extract from confluent 8701-BC and DEC-C2 cells were processed for the 2D-IPG based proteomic followed by the MALDI-TOF mass spectrometry for protein identification. To date we identified about 400 proteins, so triplicating the number of identified proteins respect to our previous data. The new proteomic evidences strengthen the anti-oncogenic effects of decorin and highlight the attention on the decreased expression of the majority of the members of three protein classes closely related to the malignant phenotype: the metabolic enzymes, the S100 family and the cell motility proteins. In conclusion, our results confirm and extend the evidence for a anti-oncogenic role of decorin and support the possibility of its use for clinical applications.

Environmental factors as possible causes of DNA fragmentation in human sperms

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Literature data demonstrated that some environmental factors could have a key role in the remarkable and continuous decline of sperm quality observed in the last fifty years. Specifically, in the Taranto area, data about the detrimental effects of environmental pollution are alarming because of the high level of poisons released in the atmosphere. Pollution coming from the plant causes health and fertility risks, mainly due to the exposure to the dioxin. Our study analyzed sperm samples from three patients groups: i) workers of local steel factories; ii) Taranto residents; iii) Controls. Results demonstrate that patients from the "factory workers" group, constantly exposed to environmental pollutants for professional reasons, show a mean percentage of DNA fragmentation above 30%. In contrast, patients from group "Taranto residents" and patients coming from Palermo considered as "Controls" group show mean percentages of 25 and 16.8%, respectively. We observed an increase of spermatic DNA fragmentation (DFI) in the "factory workers" and "Taranto residents" groups, compared to "Controls". These ones are patients of an *in vitro* fertilization clinic, with supposed fertility issues. It is known that a spermatic DFI less than 15% is physiologic, while above 30% is related to fertility issues. It is also known that interrupting the sperms damaging source might bring back the DFI level to normal values. So, moving away from the sperms damaging source, patients from "factory workers" and "Taranto residents" groups could restore spermatogenesis. The research methods employed in this study were found to be specific and valid for these analysis.

Omics approaches to elucidate the molecular physiology of lantibiotc NAI-107 production in *Microbispora* ATCC-PTA-5024

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The filamentous actinomycete *Microbispora* ATCC-PTA-5024 produces the lantibiotic NAI-107 [1], which is an antibiotic lanthipeptide effective against multidrug-resistant Gram-positive and some Gram-negative bacteria [2]. In actinomycetes, antibiotic production is often associated with a physiological differentiation program controlled by a complex regulatory and metabolic network that may be elucidated by the integration of genomic, proteomic and bioinformatic tools [3]. Accordingly, an extensive evaluation of the proteomic changes associated with NAI-107 production onset and maintenance during growth was performed on *Microbispora* ATCC-PTA-5024 by combining two-dimensional difference in gel electrophoresis, mass spectrometry and gene ontology approaches. Biomass samples were collected during growth at five time-points corresponding to different profiles of biomass and NAI-107 accumulation to perform differential proteome analyses. A total of 303 gene products, participating into 241 molecular/metabolic functions (the 14.5% of the total ones predicted from genome) were identified having a NAI-107-dependent accumulation profile. In particular, during NAI-107 production nutritional signals, regulatory cascades and primary

metabolism shift-down trigger the accumulation of protein components involved in nitrogen metabolism, cell wall biosynthesis/maturation, lipid metabolism, osmotic stress response, multi-drug resistance, and NAI-107 transport. An interesting finding was the increasing abundance of a TetR-like regulatory protein during growth progression. The over-expression of this TetR-family regulator exerted a stimulatory effect on morphological and physiological differentiation. This work, reporting the first omic-based study of *Microbispora* ATCC-PTA-5024, provides a net contribution to the elucidation of the molecular, metabolic and regulatory pathways controlling physiological differentiation and eventually leading to NAI-107 production. In addition, this study further supports the relevance and the powerfulness of proteomics in revealing novel players of antibiotic biosynthesis regulation in actinomycetes.

[1] Maffioli SI. et al. (2014) *J Nat Prod.* **77(1)**:79-84.
[2] Jabès D. et al. (2011) *Antimicrob Agents Chemother*. **55(4)**:1671-6
[3] Gallo G. et al. (2010) *Microb Cell Fact*. **26(9):**95.

Touch DNA a quarter of a century after the fact

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A broken shotgun forestock was collected at a homicide scene in 1988. The object was repeatedly scrutinized by experts in different fields in the following years, until DNA analysis was ordered in 2013. The genetic profile of a suspect and those of eight experts that had came in contact with the object were made available for comparison. Single-point swabbing methodology was used to probe the entire surface of the object; in total, 40 spots were sampled. Genotyping was performed by an ABI PRISM 3500 Genetic Analyzer with the software Gene Mapper IDX v1.3 using the PowerPlex® ESX-17 kit and AmpFLSTR NGM SElect™ Kit. In the end, 78 independent amplifications/detections of 16 autosomal STR markers were obtained. Most sample profiles were complex mixtures; however, a single major contributor was inferred in two spots; one of the two (named E1) remained unknown, the other (A18) turned out to be first- or second degree relative of the suspect with probability > 99%. Analysis of allele sharing between each of the eleven available single-person profiles (the eight experts, E1, A18, and the suspect) and all the 78 sample profiles (analytical threshold = 50 RFU) showed that, namely, two experts, A18, and the suspect, ranked highest. Suspect's profile was fully compatible with a single spot (composite method), and the likelihood ratio computed by the Forensim package for four different scenarios was 105 – 5 x 105; the corresponding value for the consensus method (four dropouts) was 103 – 3 x103. The value of the RMNE (random man not excluded) statistics for the same spot was < 10-6. For a second spot, suspect's profile showed two dropouts (composite method), and the LR for the same four scenarios was 400 – 4000. The report to the jury asserted that the data provided very strong support to the hypothesis that suspect's DNA was present on the object.

Cigarette smoke alters the DAB2IP expression in epithelial cells from COPD patients: a risk factor for lung cancer

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Cigarette smoking, one of risk factor of Chronic Obstructive Pulmonary Disease (COPD), activates epithelial cells. The alteration of the methyltransferase EZH2 has a role on the expression of the oncosuppressor DAB2IP and therefore, the cancer progression; it is often overexpressed and it promotes cell proliferation and invasion, inhibits apoptosis and enhances angiogenesis in lots of tumor. We evaluated the effect of

chronic Cigarette smoke extract (CSE) exposure on the expression of DAB2IP and EZH2 using ex vivo and in vitro studies to identify their involvement in the progression of COPD toward lung cancer. In *ex vivo* studies, EZH2 and DAB2IP expression was assessed by immunohistochemistry in bronchial epithelial cells of surgical specimens from COPD patients and healthy control subjects (HC). In *in vitro* studies, we created a chronic model using CSE; EZH2 and DAB2IP expression was studied by western blot and real time methods in bronchial epithelial cell line (16HBE) and in lung cancer cell lines (A549 and H292) stimulated with CSE. We tested the effect of GSK343, an EZH2 inhibitor, on the expression of DAB2IP and analyzed apoptosis by annexin test, and proliferation in the cell lines. Ex vivo, DAB2IP immunoreactivity was statistically significant lower while EZH2 was higher in bronchial epithelial cells (positive cells/mm^2) from COPD patients compared to HC subjects. Furthermore, DAB2IP and EZH2 expression was correlated with bronchial epithelial metaplasia in COPD. In vitro, DAB2IP was statistically significant downregulated while EZH2 increased in the cell lines CSE treated. The use of GSK343 restored the DAB2IP expression and promotes apoptosis in cells stimulated for 14 days with 20% CSE in combination with EZH2 inhibitor. Chronic inflammation due to cigarette smoke might play a critic role on the alteration of DAB2IP/EZH2 genes expression in COPD, promoting lung cancer progression toward lung carcinoma.

The cytotoxic effect exerted by parthenolide and DMAPT on breast cancer stem–like cells

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Triple-negative breast cancers (TNBCs) are aggressive forms of breast carcinoma associated with a high rate of recidivism. It is known that a small proportion of tumour cells, termed cancer stem cells (CSCs), is responsible for tumour formation, progression and recurrence. The sesquiterpene lactone parthenolide (PN) was identified as the first small molecule capable of killing CSCs.[1] Previously we have shown[2] that PN and its soluble analogue DMAPT induce a strong cytotoxic effect in MDA-MB231 cells, the most studied line of TNBCs. In the present research we investigated about the effects exerted by both PN and DMAPT on breast cancer stem-like cells derived from three lines of TNBCs (MDA-MB231, BT20 and MDA-MB436). The two compounds inhibited both the production of mammospheres from the three lines of cells and the viability of breast cancer stem-like cells derived from dissociation of mammospheres. This effect was suppressed by NAC, while z-VAD, a general inhibitor of caspase activity, was ineffective. PN and DMAPT induced in stem-like cells, in the first hours of treatment, a strong production of hydrogen peroxide. Prolonging the time of treatment (12-24h) the levels of both superoxide anion and hROS (hydroxyl radicals and peroxynitrite) increased in concomitance with down-regulation of MnSOD and catalase, dissipation of mitochondrial membrane potential and cell necrosis. It is noteworthy that treatment with PN and DMAPT also caused a rapid and remarkable decrement of the level of Nrf-2, which is a critical regulator of the intracellular antioxidant response. In conclusion PN and DMAPT markedly inhibited viability of stem-like cells derived from three lines of TNBCs by inducing ROS generation, mitochondrial dysfunction and cell necrosis.

[1] *Drug Discov Today* 2013; **18**: 894-905.
[2] *Cell Death Dis* 2013; **4**: e891.

Olive phenolics as antitumoral drug: (-)-Oleocanthal inhibits cell proliferation and induces apoptosis in hepatocellular carcinoma cells

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The beneficial health properties of Mediterranean diet is well recognized. The principle source of dietary fat in the Mediterranean diet is extra-virgin olive oil (EVOO). Oleocanthal (OLC) [(−)-deacetoxyligstroside aglycone] is a naturally occurring minor phenolic component isolated from EVOO, which showed potent anti-inflammatory activity, via its ability to inhibit cyclooxygenase enzymes COX-1 and COX-2. Since chronic inflammation plays an essential role in hepatocarcinogenesis, aim of this study was to characterize the potential anti-cancer effects of OLC in hepatocellular carcinoma (HCC). A panel of human HCC cell lines (HepG2, Huh7, Hep3B and PLC/PRF/5) was used in this study. Cells were treated with OLC and cell viability and apoptosis were evaluated. Compared with classical commercially available COXs inhibitors (ibuprofen, indometacin, nimesulide), OLC was more effective in inducing cell growth inhibition and apoptosis induction in HCC cells. OLC treatment in a dose-dependent manner inhibited cell viability in all cell lines studied, with an IC_{50} ranging from 29 μM in Hep3B cells to 75 μM in Huh7 cells at 72 hours. Moreover, OLC inhibited colony formation and induced apoptosis, as evidenced by PARP cleavage, activation of caspases 3/7 and chromatin condensation. OLC treatment in a dose dependent-manner induced expression of γH2AX, a marker of DNA damage, and increased intracellular ROS production and caused mitochondrial depolarization. OLC prevented the cell viability inhibitory effects when it was combined with rotenone, an inhibitor of electron transport chain complex. Finally, the effects of OLC are suppressed by the ROS scavenger N-acetyl cysteine. In conclusion, we found that OLC treatment exerts a potent anti-tumoral activity against HCC cells. Taken together, our findings provide preclinical support of the chemopreventive and chemotherapeutic potential of EOVV against HCC.

Differential response to high-dose radiation treatments in breast cancer cells

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Breast cancer (BC) is the leading cause of cancer-related death in women world-wide and presents distinct subtypes associated with different clinical outcomes. Radiation therapy (RT) plays a pivotal role in BC treatment often used in combination with surgery and chemotherapy. Intraoperative electron radiation therapy (IOERT) is a therapeutic technique which administers a single high dose of ionizing radiation (IR) immediately after surgical tumor removal to destroy the residual cancer cells that may be left in the tumor site. Indeed, this one typically represents a site at high risk for recurrence. The aim of this study was to highlight cell and gene expression response following IOERT treatments in human BC cells in order to find new potential biomarkers of radiosensitivity/radioresistance. We evaluated effects of the two main treatments with 9 and 23 Gy doses (*boost* and exclusive treatment for BC , respectively) through an *in vitro* approach with human mammary and BC cell lines, both immortalized and primary. Cell viability by clonogenic assay and growth curves was assessed. We observed different percentages of radioresistant cell fractions according to the cell type. DNA damage response by γ-H2AX immunofluorescence was evaluated. Gene-expression profiling using cDNA microarray allowed us to analyze transcriptional response to IOERT treatments and to identify networks and biomarkers activated in a cell type and dose delivered – dependent manner. Knowledge in the field of molecular radiobiology may provide new research approaches in order to develop

predictive tests of tumor radiosensitivity and define therapeutic treatments targeted to individual tumor subtype.

Non-canonical roles of caspase-8 in MDA-MB-231 breast cancer cell line

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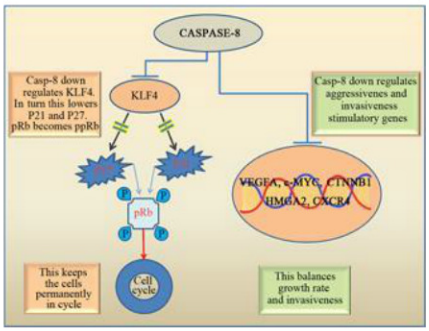
Caspase-8 (casp-8) is well known as an initiator caspase involved in cell death signalling, although its activity in many cancer cell types seems to work under non-apoptotic conditions. Moreover, in several types of cancer, casp-8 is only rarely mutated and often its expression is very elevated. Since cancer cell growth also depends on evasion of apoptosis, the upregulation of casp-8 in tumours may suggest one or more non-apoptotic roles (1). Here we report our recent studies carried out in MDA-MB-231 cells, derived from clinically aggressive forms of Triple-Negative Breast Cancer, where we have assessed the non-canonical roles of casp-8. Firstly, we evaluated casp-8 mRNA and protein levels in MDA-MB-231 cells, demonstrating that they were upregulated with respect to HMEC (normal Human Mammalian Epithelial Cells). Thereafter, to assess the role of casp-8, we silenced it by small interfering-RNA. Interestingly casp-8-knockdown, strongly decreased MDA-MB-231 cell growth by delaying G0/G1- to S-phase transition and increasing p21, p27 and hypophosphorylated/active form of pRb levels. No effects were evidenced on cell viability. To assess the metastatic capacity of MDA-MB-231 cells, the gene expression profiles of the relative markers after casp-8 knockdown were also measured. Surprisingly the expression of a number of genes and/or proteins such as VEGFA, C-MYC, CTNNB1, HMGA2, CXCR4, KLF4, VERSICAN V1 and MMP2 potently increased accompanied by migratory and metastatic capacities of cells, as shown by wound healing and matrigel assays. We suggest that among these genes, KLF4, a transcriptional factor with a dual role (activator and repressor), and responsible for p21 and p27 induction, could play critical roles (2). Casp-8 through KLF4 down-regulation, could manage the expression of critical proliferative and migratory/invasive genes. We suggest that these unusual roles played by casp-8 in MDA-MB-231 cells, should be better explored, in order to identify it as a molecular therapeutic target.



[1.] Stupack DG. Caspase-8 as a Therapeutic Target in Cancer. Cancer Lett 332:133–140, 2013.
[2.] Tiwari N et al. Klf4 Is a Transcriptional Regulator of Genes Critical for EMT, Including Jnk1 (Mapk8). PLoS One 8, 2013.

Ibuprofen containing mucus-penetrating nanoparticles as therapeutic tool for the treatment of inflammation in Cystic Fibrosis

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Conductance regulator protein (CFTR). The airways of CF patients are plugged with mucopurulent secretions containing abundant bacteria and neutrophils, and death results from progressive destruction of the lungs Cystic fibrosis (CF) is a lethal disease triggered by mutations in the gene encoding the CF transmembrane. Ibuprofen was found to significantly reduce this extreme inflammation, but despite the encouraging results obtained, in clinical the anti-inflammatory therapy is rarely practiced because of the poor penetration of drugs through mucus barrier. A novel approach could be allowed by designing particles with mucus-penetrating properties. Generally, particles with size lower than 500 nm and a neutral surface coated

by mucus inert materials are able to diffuse through pores generated by the dense fiber mesh of mucus. In this work, ibuprofen containing mucus-penetrating nanoparticles were realised starting from fluorescent derivatives of α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA), synthesized by derivatization of PHEA with Rhodamine (RhB), polylactide (PLA), and poly(ethyleneglycol) (PEG), to obtain PHEA-RhB-PLAPEG copolymers with different degrees of pegylation. Starting from these copolymers, fluorescent nanoparticles (FNPs) with different PEG content, empty and loaded with ibuprofen, were successfully prepared and showed colloidal size, slightly negative ζ potential, spherical shape and biocompatibility towards human bronchial epithelial cells (16-HBE). The presence of PEG chains and their brush-like conformation on the NPs surface was evaluated. Then, the ability of these FNPs to avoid interactions with mucus components and to penetrate CF artificial mucus (CF-AM) was properly demonstrated as a function of surface PEG density. Finally, ibuprofen release profile and uptake capacity within 16-HBE in presence of CF-AM was successfully verified.

Insulin Nanogel as New Strategy for the Treatment of Alzheimer's Disease

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A growing body of evidence shows that Insulin, Insulin Receptor (IR) and IR signaling are involved in brain cognitive functions and their dysfunction is implicated in neuronal degeneration associated with Alzheimer's disease (AD). Thus, the administration of insulin to the brain could be a strategy for the prevention and treatment of AD disease. With this aim, we have designed, synthesized and characterized a nanogel system (NG) that can be used as substrate for the conjugation of insulin and/or fluorescent molecules relevant for their characterization. In particular, a carboxyl-functionalized poly(N-vinyl pyrrolidone) nanogel system, has been produced by ionizing radiation starting from the polymeric aqueous solution and four insulin molecules per nanogel have been irreversibly attached. Absence of cytotoxicity, oxidative stress and mitochondrial dysfunction support the biocompatibility of the "naked" nanogels. Their hemocompatibility has been demonstrated by hemolysis, coagulation time, leukocyte proliferation and inflammatory response tests. By fluorescence measurements we have demonstrated that the insulin conjugated to the NG (NG-In) is protected by protease degradation and is able to bind and activate insulin receptor, thus triggering insulin signaling via AKT activation. In order to provide more significant evidence of the *in vivo* functionality of the insulin conjugated to the nanoparticles, its effect in reducing the plasma glucose levels in mice has been demonstrated. Neuroprotection of NG-In against dysfunction induced by amyloid β, a peptide mainly involved in AD, has been also verified. Finally, the potential of NG-In to be efficiently transported across the Blood Brain Barrier has been demonstrated *in vitro*. All together, these results indicate that the synthesized NG-In is a suitable vehicle system for insulin deliver and a very promising tool to develop new therapies for neurodegenerative diseases.

Cytotoxic effects of silver nanoparticles (AgNPs) biosynthesized from *Klebsiella Oxytoca* DSM29614 against breast cancer cells

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Klebsiella oxytoca DSM29614 (KO) produces a bacterial exopolysaccharides (EPSs), made of four rhamnose (Rha), two glucuronic acids (GlcA) and one galactose (Gal) bound by α and β glycosidic bonds[1,2], with metal-binding properties[3]. In particular, KO in the presence of $AgNO_3$ is able to synthesize silver nanoparticles (AgNPs) incorporated within the EPS (AgNPs-EPS). The AgNPs-EPS, may contain Ag^{+1} when KO growing in the presence of oxygen and Ag° under anaerobic conditions[4]. Currently, AgNPs are preferred to other metal nanoparticles due to its reliability and intrinsic properties such as cytotoxic and antimicrobial effects. Infact, silver is less toxic for humans when compared to other metals. In the present work were checked the cytotoxic effects of AgNPs-EPS, produced under aerobic and anaerobic conditions, on breast cancer cell line SK-BR3, monitoring: the cell proliferation inhibition rate, morphological changes and proteomic modulations. MTT assay showed significant antiproliferative activity with IC_{50} value of 5μg/ml. The most important effects were obtained by aerobically biosynthesized AgNPs-EPS treatment, due to the major release of Ag^{+1}, as verified by voltammetry analysis. Morphological alterations were consistent with apoptotic features. Proteomic analysis performed by 2D-DIGE, showed modulation of several proteins related to oxidative stress and apoptotic and mitochondrial pathways. Taken together, these results provide new important elements in support of the potential antitumoral activity of AgNPs-EPS.

[1] Leone S et al. Eur J Org Chem 2007, 31:5183-5189.
[2] Baldi F et al. J. Appl. Microbiol. 2009, 107:1241-1250.
[3] Baldi F et al. N Biotechnol 2011, 29:74-78.
[4] Battistel D et al. Talanta. 2015, 132:294-300.

Biotinylated Reduced Graphene Oxide-Based Nanocomposites for the Photothermal Treatment of Brest Cancer

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Among the relevant properties of graphene derivatives, their ability of acting as an energy-converting tool to produce heat (i.e., thermoablation and hyperthermia) was more recently taken into account for the treatment of solid tumors. In this work the in vitro graphene-induced hyperthermia was assessed and combined with the stimuli-sensitive anticancer effect of a biotinylated inulin-doxorubicin conjugate (CJ-PEGBT), hence, getting to a nanosystem endowed with synergic anticancer effects and high specificity. CJ-PEGBT was synthesized by linking pentynoic acid and citraconic acid to inulin. The citraconylamide pendants, used as pH reversible spacer, were employed to conjugate doxorubicin, whereas the alkyne moiety was orthogonally functionalized with a targeting agent (azido PEG-biotin derivative) by copper(II) catalyzed 1,3-dipolar cycloaddition. DSC measures, AFM, and UV spectrophotometry were employed to systematically investigate adsorption of CJ-PEGBT onto reduced graphene oxide (RGO) and its physicochemical stability in aqueous media, demonstrating that a stable π-staked nanosystem can be obtained. In vitro tests using cancer breast cells (MCF-7) showed the ability of the RGO/CJPEGBT of efficiently killing cancer cells both via a selective laser beam thermoablation and hyperthermia-triggered chemotherapy. If compared with the

nonbiotinylated nanosystem, including virgin RGO and the free conjugate, RGO/CJPEGBT is endowed with a smart combination of properties which warrant potential as an anticancer nanomedicine.

sample	EC50 (sec)	$EC50^{PC}$* (sec)
RGO	>300	130
RGO/CJ-PEGBT	125	105

*Obtained after an an incubation time of 24 h following the hyperthermia treatment.

Table 1. EC50 values, expressed as 810nm laser exposure, for RGO and RGO/CJ-PEGBT after 4 h of incubation followed by Laser and eventually a post incubation time of 24h. It is quite clear that the hyperthermia-induced anticancer effect in acute (thermoablation)is higher for RGO/CJ-PEGBT, if compared with the virgin RGO. This is evident comparing the EC50 values, because RGO/CJ-PEGBT had a potency at least three times higher than that observed for the virgin grapheneplatelets. A similar trend was observed after a post-incubation period, during which RGO/CJ-PEGBT inhibited cell growth more efficiently if compared with RGO alone. It can be assumed that cancer cells regrowth was avoided owing to the high amount of doxorubicin released by the nanocomposite system.

Low-Cost Synthesis of Smart Biocompatible Graphene Oxide Reduced Species by Means of GFP

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⊥*Contributed equally to this work*

The aim of this work is focused on the engineering of biocompatible complex systems composed of an inorganic and bio part. Graphene oxide (GO) and/or graphite oxide (GtO) were taken into account as potential substrates to the linkage of the protein such as *Anemonia sulcata* recombinant green fluorescent protein (rAsGFP). The complex system is obtained through a reduction process between GO/GtO and rAsGFP archiving an environmentally friendly biosynthesis. Spectroscopic measurements support the formation of reduced species. In particular, photoluminescence shows a change in the activity of the protein when a bond is formed, highlighted by a loss of the maximum emission signal of rAsGFP and a redshift of the maximum absorption peak of the GO/GtO species. Moreover, the hemolysis assay reveals a lower value in the presence of less oxidized graphene species providing evidence for a biocompatible material. This singular aspect can be approached as a promising method for circulating pharmaceutical preparations via intravenous administration in the field of drug delivery.

Management of the telemedicine system: sensors for remote monitoring of patients with chronic diseases and development of web platform to share clinical data among medical staff

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Telemedicine is the use of medical information (data, images, text, sound) exchanged from one site to another via electronic communications to improve a patient's clinical health status and can be described as: "Healthcare delivery, when the distance is a critical factor for health professionals". Telemedicine improve access to patients but it also allows physicians and health facilities to expand their reach, beyond their own offices. Today, this discipline is widely able to break down geographical barriers giving remote support "in real time". Reducing or containing the cost of healthcare is one of the most important reasons for funding and adopting telehealth technologies. Our aim is to improve the quality of healthcare in chronical disease through the constant monitoring of vital signs using wearable device,. For this purpose, we have evaluated and used devices that have the ability to record and analyze the following biometric parameters: ECG and heart rate, respiratory rate, oxygen saturation, posture. An important function of these devices is to generate an alert, when the values exceed the normal range. Our research was focused on the evaluation of the functions of the devices, at IBIM CNR, and applied in 12 control and 12 patients with heart disease, at ARNAS Civico Hospital. The biometric signals detected were transmitted, through a wireless system, in an online repository called Electronic Health Record and stored there. This platform represents an important resource software, able to manage the sharing of data collected and processed by different equipes, allowing to trace a specific clinical profile for each monitored patient. The platform allowed health professionals, signed up, to retrive the data and different professionals in different geographic areas to access the data (Second Opinion).

The Salt Inducible Kinase 2 (SIK2) links lipid metabolism to survival of ovarian cancer metastasis

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Background: High Grade Serous Ovarian Cancer (HGSOC) metastatic disease is strongly dependent on the adipocyte-rich microenvironment of the omentum. We have previously shown that expression of the Salt Inducible Kinase 2 (SIK2) is important for ovarian cancer cell survival. In this work we present a previously unrecognised role for SIK2 in driving cancer cell metabolism and proliferation at the omental metastatic niche. **Methods:** A system for the co-culture of cancer cells with adipocytes obtained from freshly excised normal omentum was used in combination with a chemical biology approach utilizing cells expressing gatekeeper mutants and a type I kinase inhibitor. Our results were validated using immunohistochemistry of a panel of ovarian cancers and a mouse model of ovarian cancer metastasis. **Results:** SIK2 was significantly overexpressed in omental metastases of ovarian tumours compared to paired ovarian cancer primary lesions from the same patients. In a xenograft model of ovarian cancer metastasis SIK2-overexpressing cells implanted orthotopically at the ovarian bursa formed significantly larger omental metastases compared to cells with endogenous levels of SIK2. Surprisingly, we observed that co-culture of ovarian cancer cells with adipocytes resulted in an increased SIK2 autophosphorylation and activation which, in turn, stimulated cancer cell metabolism via phosphorylation of ACC1 and survival throught activation of PI3K pathway. **Conclusion:** Our results suggested that SIK2 phosphorylation and activation were required to establish ovarian cancer lesions at the adipocyte-rich omental environment. Therefore we suggest a therapeutic role for targeting SIK2 in preventing ovarian cancer metastases.

Bryophytes: a powerful source of biologically active compounds

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Bryophytes are generally considered the first plants appeared on the Earth. Taxonomically, they are placed between Algae and Pteridophytes. The medicinal traditional use of bryophytes has been documented for a long time in many countries. Bryophytes contain numerous potentially useful compounds and strong antioxidative enzymatic machinery, which help them to cope up with extreme climates and stresses, and, in several cases, the total antioxidant amount was found higher than in other antioxidant common sources even if not yet fully known. Thus, a positive correlation between the folklore use and the scientific evaluation could generate an alternative source of novel medicinal compounds, which might overcome the expensive production of synthetic drugs and their undesirable or long-term side effects. It is known that plant antioxidants may have beneficial health effects. Reactive Oxygen Species (ROS) are formed as by-products of metabolic reactions in living organisms. If unbalanced, they initiate toxic oxidative reactions leading to oxidation of proteins, lipids and nucleic acids, thus triggering pathological conditions such as neurodegeneration, inflammation, aging process, cancer and cardiovascular diseases. Recent pharmacological investigations have demonstrated that the active principles present in bryophytes are quite unique for their potential therapeutic applications. The main purpose of the present work is to test the activity of these molecules on the oxidative cell processes.

PCL/PEG based membranes for bacterial cells immobilization stimulate actinorhodin antibiotic production in S. coelicolor

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The actinomycetes, Gram-positive filamentous bacteria, are the most prolific source of natural antibiotics. At industrial level, antibiotics from actinomycetes are usually produced at low levels by mean of fermentative processes in submerged cultivations, where one of the major factors negatively affecting bioproductivity is the phenomenon of a pellet-shaped biomass growth. The strategies based on cell-immobilization, which were already proven improving bacterial bioprocess productivity, could stimulate antibiotic production in actinomycetes. Accordingly, polycaprolactone (PCL)/polyethylene glycol (PEG)-based porous membranes, having pore sizes of 50, 100 and 500 μm, were used for the immobilization of Streptomyces coelicolor, a model strain for studying actinomycete biology. Using 96-well microtiter for bacterial cultivations, the immobilized S. coelicolor cells formed a dense layer on pore lumen of all PCL/PEG membranes tested as revealed by scanning electron microscope. In particular, the actinorhodin and biomass production yields were strongly pore-size dependent with the 100 μm sized pores showing the best performance in the respect of free cell cultivations. Similar results were obtained using tester tubes instead of 96-well microtiters too. Therefore, microporous PCL/PEG membranes could be useful tool for antibiotic production improvement in actinomycete-based bioproduction processes. In addition, they can be exploited to study the biochemical and metabolic events which lead to the improvement of bioproductivity in immobilized bacterial cells.

Blue Biotechnology and Cultural Heritage: case studies

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In order to promote innovative methodologies for conservation and restoration of historic-artistic manufacts, the efforts are focalized on the development of "*sustainable*" applications as alternatives to traditional restoration procedures, which can sometimes be detrimental for the artworks, humans and environment [1]. The role of Biotechnology in this field showed very useful applications for diagnosis of bio-deterioration of cultural assets, by an integrated methodology based on molecular and microbiological skills, and in bio-cleaning/bio-removing of organic/inorganic layers from artwork surfaces by enzymes or viable bacteria cells [2, 3]. In this work, bioactive molecules isolated from marine organisms were utilized for enzymatic removal of aged/degraded layers (waxes, re-paintings, glued paper, protective layers, consolidating products) both from laboratory specimens or artworks surfaces (paintings, mosaics, wax statues). Particularly, biocleaning protocols were carried out using bioactive molecules with Protease and Esterase activity. The enzymes were utilized in water solutions gelled by Klucel-G or Carboxymethyl-cellulose gelling–agents, guaranteeing a controlled and selective action. These novel enzymes showed important advantages: they are active at temperature lower than 30°C, they need a reduced time of application (10-20 minutes), are safety for both operators and environment [4]. In our hypothesis, these molecules provide an important contribution to the development of sustainable innovative protocols.

[1] Tomei F, Baccolo TP, Papaleo B, Biagi M, Signorini S, Persechino B, Rosati MV (1996). Effects of Low-Dose Solvents on Blood of Art Restorers. Journal of Occupational Health, 38: 190-195.

[2]Palla F (2013). Bioactive molecules: innovative contributions of biotechnology to the restoration of Cultural Heritage. Conservation Science in Cultural Heritage, 13, 369-378.

[3] Ranalli, G., et al. (2005). Biotechnology applied to cultural heritage: biorestoration of frescoes using viable bacterial cells and enzymes. Journal of applied microbiology 98, 73-83.

[4] Barresi G, Di Carlo E, Trapani MR, Parisi MG, Chillè C, Mulè MF, Cammarata M, Palla F (2015). Marine organisms as source of bioactive molecules applied in restoration projects. Heritage Science, 3 (3), 17-20.

Bioremediation of oil-contaminated water using scaffold-bacterial biofilm systems

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Bioremediation is a promising non-invasive and cost-effective technology that uses (micro)organisms to degrade or remove hazardous environmental pollutants. New methods are needed to enhance and optimize natural biodegradation, such as the use of carrier materials that could improve survival and catalytic activity of the biodegraders. In this study, we developed a bioremediation system based on a new 3D polycaprolactone-based scaffold and hydrocarbon(HC)-degrading bacteria to clean (sea)water contaminated by crude oil and its derivatives. Scaffold biopolymers are biodegradable, produced in the melt, i.e. at low cost and without the use of toxic solvents. They can be available in large quantities and are endowed with a marked lipophilicity[1]. The bioremediation efficiency of our system was tested on crude oil and *n*-alkanes using two highly performant HC-degrading bacterial strains: the marine hydrocarbonoclastic model strain *Alcanivorax borkumensis* SK2 and the soil long-chain *n*-alkane degrader *Nocardia* sp. SoB. A high capacity of adhesion and proliferation of bacterial cells within the whole three-dimensional structure was observed using scanning electron microscopy. The bacterial degradation ability of HC-embedded scaffold was

evaluated by GC-FID analysis. Total oil HC degradation rates of ~50% and ~40% of were observed after 6 days incubation, for *Nocardia* and *Alcanivorax*, respectively; rates of biodegradation of ~90% (*Nocardia*) and ~60% (*Alcanivorax*) were observed for *n*-alkanes after the same incubation period. The degrading ability of the scaffold-bacterial cells-system was compared whit that of free-living cells. The use of this bioremediation system may lead to a better interaction between the hydrophobic substrate and the bacterial cells, increasing the bacterial degradation ability.

POSTERS

Sviluppo e Differenziamento (SD)

SD1
The autophagic demand during oogenesis and early development of sea urchin

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The autophagic pathway is an evolutionarily conserved homeostatic process, responsible for degradation and recycling of long-term proteins and cytoplasmic organelles in eukaryotic cells. This process constitutively occurs at basal levels and is involved in cell survival. Increased autophagy is induced by environmental cues, such as starvation and many stress agents, while excessive levels of autophagy can lead to autophagic Programmed Cell Death, with features that differ from those of the apoptotic process. We recently demonstrated massive activation of autophagy in *P. lividus* embryos, in cadmium stress conditions, and the existence of a temporal relationship between induced autophagy end apoptosis. Although there are numerous studies on the role of autophagy during development of different organisms, only a few of them examine its role during oocyte maturation and early embryogenesis. Here we report our recent data about the occurrence of autophagy, from oogenesis to early stages of embryogenesis. By detection and quantification of autophagic markers i.e. acidic vesicular organelles (AVOs) and LC3-II protein we observed that: i) oocytes and early developmental stages exhibit a peculiar localization of autophagic signals; ii) these signals greatly increase after fertilization until 32 blastomeres; iii) interestingly a short autophagic inhibition, after fertilization, strongly interferes with starting developmental program, causing evident and irreversible impairments of morphology, as well as induction of apoptosis at gastrula stage. These data lead to the hypothesis that autophagy could act: i) during oogenesis, for recycling of cellular components and for elimination of the germinal vesicle; ii) during early embryogenesis, for yolk digestion or for removal of obsolete proteins and organelles; iii) in the micromeres for some mechanism probably linked to the subsequent gastrulation process. Altogether these considerations pave the way for further investigation on the role of autophagy during development.

SD2
Evaluation of Epithelial to Mesenchymal Transition in *P. lividus* embryo

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Epithelial to Mesenchymal Transition (EMT) is an evolutionarily conserved developmental process which is essential for shaping embryos, but it is also a key step in activating carcinoma cell motility and metastasis (1). During the EMT process the typical architecture of epithelia undergoes changes, cells loose cellular contacts and increase their migratory ability, acquiring a mesenchymal phenotype. In sea urchin embryos, early EMT is related to the physiological migration of primary mesenchyme cells (PMC) into the blastocoele where later they give rise to the embryonic skeleton. In preliminary screenings, treatment of *P. lividus* embryos from fertilization up to 16 hr with different concentrations of Kenpaullone (9-bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one), led to the development of empty blastulae, suggesting that Kp did not inhibit cleavage *per se*, but prevented EMT. Embryos treated at hatching blastula stage were instead more robust. Kp, a synthetic molecule, was described as an ATP-competitive kinase inhibitor of *GSK3* and *CDKs*(2). In order to define embryonic sensitivity to Kp, linked to specific developmental stages, we added the drug at different concentrations immediately after fertilization for 10 minutes; Kp was then removed and embryos let to develop. Mesenchyme cells differentiation was strongly perturbed, as manifested by aberrant development and the lack of expression of the mesenchymal-specific markers.

Moreover, the expression of two genes, *WNT8* and *GSK3,* involved in EMT was evaluated. In Kp treated embryos from 8-cell to early blastula stages *Wnt8* gene expression was down-regulated, while *GSK3* levels did not change in treated and untreated embryos. At late gastrula stage, *Wnt8* expression level was recovered and *GSK3* was over-expressed, but the altered phenotipe did not revert. These results indicate that Kp treatments at very early cleavage stages severely perturb the PMCs specification and the EMT process in a irreversible manner.

[1] Lim J and Thiery JP. Epithelial-mesenchymal transitions: insights from development. Development. 2012 Oct;139(19):3471-86. doi: 10.1242/dev.071209.

[2] Tolle N. and Kunick C. Paullones as inhibitors of protein kinases. Currents Topics in Medicinal Chemistry. 2011;11(11):1320-32.

SD3

Phylogenetics of *Anadenanthera colubrina* (var. *cebil*) tree from Salta (Northwest Argentina)

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A. colubrina var. *cebil* (Vell.) Brenan represents a trace in memory of the forests that decreased over the years and this is the reason why it's interesting to study and to preserve it. This is an important tree for cultural, economic, and medicinal uses in South America. In order to implement a policy of conservation of *A. colubrina*, we have characterized the genetic diversity of 4 populations. We collected seeds from 4 different sites (B San Bernardo, C El Cebilar, M Metán and G El Gallinato) in Salta Province. Then, we compared the intergenic transcribed sequences (ITS) of ribosomal DNA (rDNA), a known molecular marker. Our previous results (1), obtained through morphological and genetic analysis of only 4 individuals (one per zone), have showed that B and M individuals were more similar each other, as well as G and C. Here, a largest number of individuals (29) were characterized, and their phylogenetic relationships analysed. The results comfirmed the previously found similarities, indicating that the genetic characteristics of *A. colubrina* populations follow a geographic distribution, probably related to the environmental climate. Besides, we found that populations of *A. colubrina* from the Salta province showed high genetic distance and different haplotypes in ITS-rDNA, that could be a consequence of different colonization events. This finding is expected in forest trees due to high population sizes, longevity of individuals, and high levels of gene flow between populations. As tree have a crucial role in the social, ecological and economic life, covering the 30% of Earth, the study of plant diversity may help conservation of forests.

SD4

A new molecular approach for shelf-life evaluation of the crustacean *Nephrops norvegicus* (Linnaeus, 1758)

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The European lobster *Nephrops norvegicus* is an appreciated seafood with a relevant economic value in the crustacean business sector. Many factors as environmental pollution, catching/handling stressors or improper management of the cold chain may adversely affect quality and shelf-life of crustaceans. Fishing industry and trading companies are strongly requiring new scientific tools, as potential support in seafood management to get quality and competitiveness. In this work, we developed an innovative molecular approach to assess the shelf-life of the lobster *N. norvegicus.* For the first time, we investigated muscle exudates of thawed specimens as an easy, potential source of nucleic acids. Despite their hypothesized degradation, we were able to detect specific nucleotide sequences. At NCBI database, we selected *N. norvegicus* mRNAs sequences as potential indicator(s) of molecular effects (stress/immune responses and/or

degradations) of biological events linked to catching, handling, and freezing/thawing cycles. We obtained small amplicons of Actin, Cytochrome Oxidase I (COI), Calpain M, Prophenoloxidase (ppo) and Crustin-like antimicrobial peptide, in preliminary One Step RT-PCR using polyA+ RNA or total RNA. By comparative One Step RT-PCR and/or QRT-PCR assays we evaluated the amplification efficiency (AE) of the selected mRNAs using total RNA extracted from muscle exudates of thawed specimens, maintained at 4°C for 1, 4 and 7 days (shelf-life). We found that long shelf-life and lowest quality correlate with the lowest values of AE. Our results provide new opportunities in food science applications and in industrial-scale management, to track quality and shelf-life over time.

SD5
In silico analysis of Kibra and STK4 as main components of Hippo Pathway in Sea Urchins

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Hippo pathway controls organ size through the regulation of cell proliferation and apoptosis. Perturbation of upstream components of the Hippo pathway leads to tissue overgrowth and enlarged organ size without major changes in organ patterning. First discovered in *Drosophila*, many of Hippo pathway components have been identified in mammals. At the central core of the signalling cascade are MST1/2 kinases, two Hpo homologs in mammals. Mst1/2 are part of a conserved kinase cassette that, regulating downstream transcription coactivators YAP and TAZ, promote tissue proliferation, self-renewal of normal and cancer stem cells, migration, and carcinogenesis [(1, 2)]. Recent studies have identified KIBRA (WWC1) as an upstream regulator of the Hippo pathway in *Drosophila* and mammals. Kibra/KIBRA is a cytoplasmic protein that acts by binding to Mer/NF2, and their co-expression results in synergistic phosphorylation of Warts (Lats1/2) [(3, 4)]. We have identified orthologous candidate Kibra and STK3/4 (MST1/2) proteins in the sea urchin *Strongylocentrotus purpuratus*. Sea urchin predicted Kibra and STK4 contain canonical domains and share protein structures with the *H. sapiens* and *D.melanogaster* counterparts. Multiple sequence alignments were showing high identity scores in both protein and nucleotide sequences between *S. purpuratus* and other species. The analysis of intron-exon organization between *S. purpuratus* and *Paracentrotus lividus* (Mediterranean sea urchin) *Kibra* and *STK4* genes, allowed to determine that genomic structure is well conserved between the two species. Analyses obtained by the estimation of the derived phylogenetic trees, showed that sea urchin Kibra and STK4 were highly similar to related proteins of the Deuterostome group.

[1] Pan D.. The hippo signaling pathway in development and cancer, *Dev. Cell* 19 (2010) 491–505.
[2] Zhao B., Tumaneng K., Guan K.L.. The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal, Nat. *Cell Biol.* 13 (2011) 877–883.
[3] Yu J., Zheng Y., Dong J., Klusza S., Deng W.M., Pan D. (2010). Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. *Dev. Cell* 18, 288-299
[4] Zhang N., Bai H., David K.K., Dong J., Zheng Y., Cai J., Giovannini M., Liu P., Anders R. A., Pan D. (2010). The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. *Dev. Cell* 19, 27-38

SD6
Developmental abnormalities induced by Gadolinium causes a time-dependent miss-expression of regulative and structural genes in P. lividus sea urchin embryos

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Gadolinium (Gd) is a metal of the lanthanide series of the elements whose chelates are commonly used as contrast agents for magnetic resonance imaging. Its release into the aquatic milieu has posed serious concerns regarding its noxious effects, and therefore Gd is now considered an emerging environmental pollutant. The sea urchin embryo is an excellent model used in both toxicological and developmental research. We analysed the consequences of embryo exposure to sublethal concentrations of Gd on embryo development, focusing on skeletogenesis and developmental symmetry. We observed a strong inhibition of skeleton growth, frequently displayed by an asymmetrical pattern. Continuous exposure to Gd of sea urchin embryos caused autophagy, but not apoptosis. Results showed an increase of the LC3 protein at 24 and 48h, confirmed by the increased number of autophagosomes and autophagolysosomes observed by confocal microscopy. RT-PCR gene expression analysis showed the misregulation of several genes acting at different functional and hierarchical levels of both the skeletogenic and the left-right axis specification networks. These included: transcription factors (Alx-1, Nodal), signaling molecules (univin, VEGF, VEGF-R, FGF) and skeletal matrix proteins (p16, p19 and msp130). Embryos were exposed to the same Gd concetrantion and harvested at 6, 24 and 48 hrs post fertilization (hpf). After 24 hpf, Alx-1 and Nodal showed respectively 40% and 60% reduction of their relative transcriptional levels, while only Alx-1 was reduced by 60% at 48 hpf. A 50% reduction of univin, msp130 and p16 was found at 48 hpf, while FGF was reduced by 60%. Taken together, the results pose serious questions on the hazard of Gd in the marine environment and indicate that Gd is able to affect three different levels of the stress response in sea urchin embryos: morphogenesis, survival strategies such as autophagy, and gene expression.

SD7
Identification of a putative GAGA factor in *P. lividus* embryos

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Drosophila GAGA factor, (GAF), encoded by the gene *Trithorax-like* (*Trl*), initially identified as a regulator of developmental genes (Hox gene), has been subsequently shown to be involved in different processes: chromatin remodelling, "Polycomb responsive element" function and insulator/boundary functions (1,2). Multifactorial gene regulation by GAF has been attributed to its ability to recognize and specifically bind to GAGAG consensus DNA motif by its C2H2 zinc finger domain and to interact with other regulatory factors by its BTB/POZ domain. The roles it plays seem to be conserved along evolution but its vertebrate orthologue, c-KROX, has been only recently identified and characterized (3). We have now evidence of the presence of a putative GAGA factor in sea urchin and we are investigating its role in the regulation of the expression of the *P.lividus* E- histone genes cluster. GAGA sites have been shown to be necessary for the correct temporal expression, during embryogenesis, of early histone genes and for the function of the sns5 chromatin insulator present in the cluster (4). We have identified, by in silico analysis, a factor which shares with Drosophila and vertebrate GAGA factor the presence of both N-terminal BTP/POZ and C2H2 Zinc finger domains. By RT-PCR we have isolated a 2.5kb cDNA corresponding to the entire coding region. One step RT-PCR, performed with RNA from developing embryos, has revealed that this factor is always expressed until larval stage. An antibody has been raised against the putative GAGA factor and its specificity

assessed by western blot. Finally, we have performed ChIP experiments whose results strongly support the hypothesis that this new factor can bind to the sns5 insulator.

[1] Srivastava S et al. 2015 Available online
[2] Matharu NK et al. 2010 JMB 400 (3): 434-447
[3] Fuda NJ et al. 2015. PLoS Genet 11(3): 100-108
[4] Melfi R et Al. 2001 JMB (1); 304:753-63

SD8

The nucleic acid-binding protein *PcCNBP* is transcriptionally regulated during the immune response in red swamp crayfish *Procambarus clarkia*

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Cellular nucleic acid binding proteins (CNBPs) represent a highly conserved protein family among vertebrates; they harbour seven tandem zinc finger repeats CCHC type and have been described as transcriptional and translational regulator. To date, there is little characterisation of CNBP in invertebrates since its structure and function have been analyzed solely in *Drosophila melanogaster.* However no CNBP has been investigated in other arthropod systems. In an effort to isolate immune-related genes in *Procambarus clarkii,* a partial mRNA coding a zinc finger containing protein was found to be up-regulated during the response to white spot syndrome virus (WSSV) infection.. The red swamp crayfish *P. clarkii*, represents an attractive animal model because of its tolerance to extreme environmental conditions and resistance to diseases. Thus it has become an important crustacean model organism for virological studies. In this study, a CNBP homolog from the red swamp crayfish *Procambarus clarkii* was characterised. The full-length cDNA of *PcCNBP* was of 1257 bp with a 5'-untranslated region (UTR) of 63 bp and a 3'-UTR of 331 bp with a poly (A) tail, and an open reading frame (ORF) of of 864 bp encoding a polypeptide of 287 amino acids with the predicted molecular weight of about 33 kDa. The predicted protein possesses 7 tandem repeats of 14 amino acids containing the CCHC zinc finger consensus sequence, two RGG-rich single-stranded RNA-binding domain and a Nuclear localization signal, strongly suggesting that PcCNBP was a homolog of vertebrate CNBP. Analyses of transcriptional expression profile showed that *PcCNBP* was constitutively expressed among different tissues from of the adult crayfish, under normal physiological conditions. Moreover, qRT-PCR assays indicate that the transcriptional expression of *PcCNBP* responds to bacterial and viral stimulations.

SD9

The analysis of the HSA20/21 Syntenic Association in Cercopithecini allows a Discussion on Neocentromeres Scattering in Primate Genomes

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In spite of many expectations there is not any room for the concept that mammalian genomes have a default chromosome rearrangement rate, and that sister taxa have an intelligible and predictable chromosome organization. A part from the initial distress, these evidences resulted very stimulating for researchers. *In situ* hybridization studies conveyed to a series of proposed "Ancestral Karyotypes", and to the consequent discussion about, for example, the "conservativeness" of Carnivora, the rapid chromosomal evolution in Perissodactila or in Rodentia and Primates (Supraprimates/Euarchontoglires). Chromosome painting and BACs FISH identified a series of apomorphic syntenic association in primates. Cercopithecini Tribe (Anthropoidea, Cercopithecoidea) is characterized by an apomorphic HSA20/21 syntenic association. This association demonstrates a high rate of polymorphism. We analyzed several species in the wide distributed African tribe of tree-dwellers, often identifying the 20/21 association as an heteromorphic pair in the

karyotype. Further, five different centromere position have been recognized inside this syntenic association in the different species. These evidences justify the hypothesis that, a part from pericentromeric inversions, the heteromorphy could be sustained also by the activation of neocentromeres distributed along the chromosomes. Here we discuss the origin and development of this peculiar trans-specific heteromorphy in the light of molecular cytogenetics and bioinformatics analysis of human chromosomes 20 and 21, and of other syntenic association of the homologous 21 in Primates.

SD10
The interaction of a cobalt(II) Schiff base complex with duplex-DNA and G-quadruplex DNA

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Since the discovery of the binding of cis-dichloro-diamine-platinum(II) (cisplatin) to duplex-DNA, the interactions between small molecules and nucleic acids has gained high interests among scientific community for their involvements in medicine, especially in anticancer treatments. Nevertheless, duplex-DNA is a ubiquitous target and therefore such drugs always carry several serious side effects. To improve drug selectivity, novel targets have been considered, such as proteins and, remarkably, non-canonical secondary structures of the polynucleotides, especially guanine-quadruplexes. The latter has been recently recognized in telomeric DNA (h-Telo) where the G-quadruplex inhibits the activity of telomerase, thus arresting uncontrolled proliferation of cancer cells. Moreover, guanine-enriched sequences have also been found in promoter regions of many proto-oncogenes, playing a key role in gene-transcription processes. Transition metal complexes of Schiff bases have shown to be effective G-quadruplex stabilizers, leading to senescence and apoptosis of cancer cells. Herein we report the synthesis of a novel cobalt(II) Salphen-like complex and its interaction with both duplex and G-quadruplex DNA, from telomeric and c-Kit protooncogene sequence

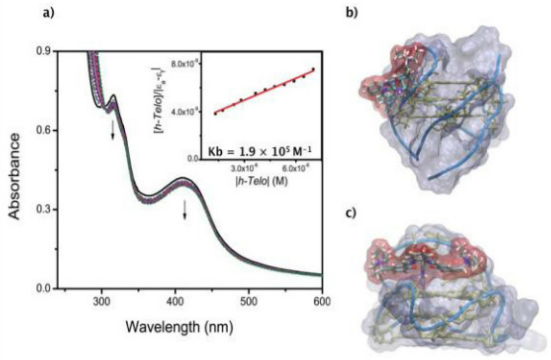

Figure 1. (a) Example of UV-Vis absorption spectra of the cobalt(II) complex in the presence of increasing amounts of G-quadruplexes from h-Telo; structural models of the DNA-binding interaction with h-Telo (b) and c-Kit (c) sequences. The investigations, carried out by means of UV absorption spectroscopy, circular dichroism and viscosimetry, have shown the metal complex is a grove binder for both duplex both G-quadruplex conformations. In particular, affinity constants (Kb) towards h-Telo and c-Kit are about 10 fold higher than that for B-DNA, showing selectivity of the synthesised complex. Biological assays are presently ongoing to assess the antiproliferative activity of the cobalt complex.

SD11
MiRNATIP: miRNA-Target Interaction Predictor

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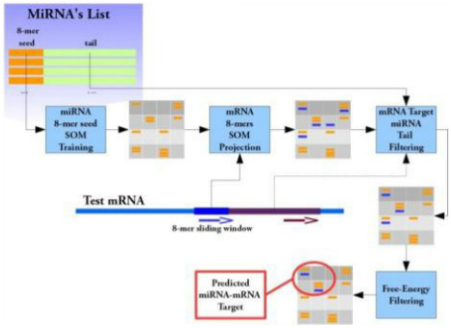
MicroRNAs (miRNAs) are small non coding RNAs with regulatory functions to post-transcriptional level. They play an important role in molecular and cellular mechanisms, thanks to their ability to bind and interact with many RNA messengers (mRNAs) of coding product involved in a wide range of biological pathways, cellular status, and conditions. We present miRNA Target Interaction Predictor (miRNATIP), a Self

Organizing Map (SOM) based method for the miRNA target prediction. miRNATIP is composed of four steps (see Fig. 1): in the first step, a set of miRNA seeds (8 nt) is used for the training of a SOM. The second step is the projection of a mRNA sequence over the trained SOM. For this reason, we extracted all the 8-length mRNA fragments through a 8-mer sliding window. The result of this step is, for each neural unit (cluster), a list of miRNA_seed-mRNA_fragment. Each cluster can be considered as a preliminary list of predicted miRNAs-mRNAs interaction. Then we computed a dissimilarity measure based on normalised euclidean distance between the remaining part of both miRNA and mRNA sequences, and we retained only the couples whose distance is below a certain threshold. Finally, in the fourth step we performed another filtering to the miRNA-mRNA interaction list, by computing the free-energy of the miRNA-target site duplex. We tested our method by predicting the miRNA target interactions of the C. elegans and human species. miRNA mature sequences were downloaded from miRBase, while verified 3'UTR mRNA sequences were extracted from Ensembl. Experimentally validated miRNA-target interaction were taken from mirTarBase and Tarbase. We compared our results with other target predictors: PITA, miRanda, TargetScan, Pictar, Diana-microT. Prediction results, in terms of sensitivity and specificity, demonstrated that outperforms or is comparable to the other six state-of-the-art methods, in terms of validated target and non-target interactions, respectively.



Malattie Metaboliche (MM)

MM1

Production of hyaluronic acid derivative microfibers with controlled dimension as potential drug delivery system

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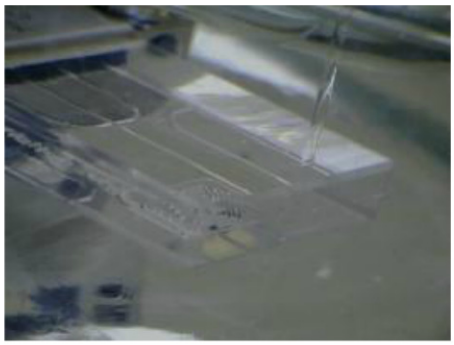
Microfluidic generation of continuous polymer fibers has many advantages such as easy control of size, simple fabrication process of microscale fibers, and easy loading of biological molecules. Polysaccharides and their derivatives are suitable candidates for modified drug release systems. This work reports the production of microfibers based on a hydrophobic derivative of hyaluronic acid (HA-EDA-C18) loaded with dexamethasone using a microfluidic technique. The introduction of octadecylamine (C18) and ethylenediamine (EDA) portions on hyaluronic acid backbone made the new derivative soluble in water but sensible to ionic strength [1,2]. This particular behavior has been employed to obtain physically crosslinked microfibers immersing the chip in phosphate buffer solution without using chemical crosslinkers. Obtained microfibers have been characterized with optical microscopy and SEM analysis to evaluate their morphology after production in different coagulating media and after one week in phosphate buffer solution, to check their stability. Drug loading and release from microfibers have been also studied, by using dexamethasone as a model drug. This study has showed a simple, cost-effective, well-controlled and biologically compatible process for the production of uniform HA-EDA-C18 microfibers with controlled size and morphology, able to prolong dexamethasone release.



Picture of fabrication process of HA-EDA-C18 microfibers.

[1] F. S. Palumbo et al.,(2015) Carbohydr. Polym, 122, 408–416.
[2] F. S. Palumbo et al., (2015) RSC Adv., 5, 61440-61448.

MM2

The chronic treatment with a diet supplement containing *Curcuma longa,* guggul, silymarin and chlorogenic acid counteracts the development of NAFLD and atherosclerosis in a mouse obesity model

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Non-alcoholic fatty liver disease (NAFLD) confers an increased risk of cardiovascular disease. A leading hypothesis is that hepatic steatosis may lead to the production of pro-inflammatory cytokines, which accelerate atherosclerosis process and lead to progressive vascular dysfunction. Furthermore, Angiotensin II (Ang II) production, starting from the hepatic precursor angiotensinogen (AGT), seems to play an important role in atherogenic plaque initiation and growth. Using a mouse model of diet induced-obesity (DIO) that develops NAFLD, the impact of a natural diet supplement (kepar, Rikrea, Italy) containing plant extracts such as *Curcuma longa,* guggul and chlorogenic acid on liver function and atherosclerosis plaque formation was evaluated. Animals, fed high fat diet, were divided into two groups, whose one was treated with oral administration of kepar (1.6 g/die) for 4 months. Expression of genes related to the hepatic dysfunction (Profiler PCR array), plasma Ang II levels (ELISA), hepatic AGT-RNAm expression (RT-PCR), liver steatosis, aortic plaque development and carotid artery thickness (histological analysis), were evaluated and compared to untreated group. In kepar- treated group, the array profile of the liver showed pro-inflammatory mediator downregulation and lipolysis gene upregulation. In treated mice, the liver steatosis was reduced, as well as the AGT-mRNA expression and Agt II plasma levels and histological analysis showed neither atherogenic vascular lesions nor carotid artery thickening. The present study highlights the cooperative action of plant extracts present in kepar as well as the anti-inflammatory properties, resulting protective not only in NAFLD but also against atherogenesis development.

MM3

Management of the telemedicine system: sensors for remote monitoring of patients with chronic diseases and development of web platform to share clinical data among medical staff

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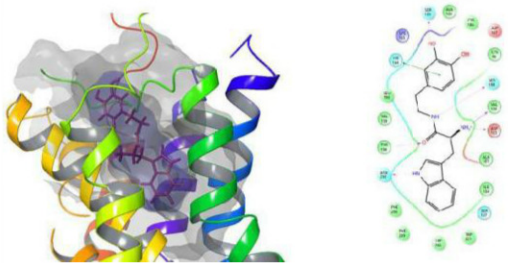
Telemedicine is the use of medical information (data, images, text, sound) exchanged from one site to another via electronic communications to improve a patient's clinical health status and can be described as: "Healthcare delivery, when the distance is a critical factor for health professionals". Telemedicine improve access to patients but it also allows physicians and health facilities to expand their reach, beyond their own offices. Today, this discipline is widely able to break down geographical barriers giving remote support "in real time". Reducing or containing the cost of healthcare is one of the most important reasons for funding and adopting telehealth technologies. Our aim is to improve the quality of healthcare in chronical disease through the constant monitoring of vital signs using wearable device,. For this purpose, we have evaluated and used devices that have the ability to record and analyze the following biometric parameters: ECG and heart rate, respiratory rate, oxygen saturation, posture. An important function of these devices is to generate an alert, when the values exceed the normal range. Our research was focused on the evaluation of the functions of the devices, at IBIM CNR, and applied in 12 control and 12 patients with heart disease, at ARNAS Civico Hospital. The biometric signals detected were transmitted, through a wireless system, in an online repository called Electronic Health Record and stored there. This platform represents an important

resource software, able to manage the sharing of data collected and processed by different equipes, allowing to trace a specific clinical profile for each monitored patient. The platform allowed health professionals, signed up, to retrive the data and different professionals in different geographic areas to access the data (Second Opinion).

MM4

Molecular modeling studies on dopamine-amino acid conjugates as potential dopaminergic modulators

A. Chinnici[1,2], A. M. Almerico[1], M. Tutone[1]



DA-Trp interactions with D1DR after Induced Fit Docking analysis.

In the last years, prodrug strategy was used with the aim to increase drugs selectivity especially in CNS reducing systemic and/or organ-specific toxicity. The dopamine-amino acid conjugate DA-Phen was first designed in order to obtain a useful prodrug for Parkinson's disease therapy, but experimental evidence shows that it effectively interacts with D1 dopamine receptors (D1DRs), leading to an enhancement in cognitive flexibility and to the development of adaptive strategies in front of an aversive environment[1]. In the attempt to identify new compounds with potential dopaminergic activity, we performed a molecular modeling study on other dopamine conjugates. In order to find a method able to give the best predictions of the D1DR binding, we used three different approaches. Molecular Dynamics (MD) simulation was first carried out to analyze D1DR behavior during the interaction with DA-Phen. Cluster Analysis of MD trajectory snapshots was then employed to select the most significant conformations to be used in semi-flexible docking with known agonists and antagonists. Then, we performed semi-flexible docking on the original model, and Induced Fit Docking (IFD). This last method gave the most interesting results, therefore it was the preferred one to perform computational studies on new DA conjugates. Other 19 dopamine-amino acid conjugates were screened. IFD poses of the new conjugates were used to perform MM-GBSA analysis to calculate the ΔG binding energy values, and the most promising compound resulted DA-Trp, followed by DA-Leu and DA-Pro. On the basis of these results, we deem that DA-Trp, DA-Pro and DA-Leu could be potential D1DR agonists, suggesting a possible use for further in vivo studies.

[1] De Caro V., Sutera F.M., Gentile C., Tutone M., et al. Studies on a new potential dopaminergic agent. In vitro BBB permeability, in vivo behavioural effects and molecular docking. J. Drug Target. 2015, 10, 910-925.

MM5

Ex-Vivo model for the evaluation of drugs and micellar systems permeation across cornea

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A.

Nowadays, diseases affecting posterior eye segment are increasing at an alarming rate. These include age-related macular degeneration, diabetic macular edema and diabetic retinopathy. Currently, the intravitreal administration is widely used, even if frequent injections can lead to retinal detachment, endophthalmitis and increased intraocular pressure. To overcome these problems, the topical administration of nanotechnology-based drug delivery systems is a strategy presently used. In particular, polymeric micelles are proposed as an

effective carrier to transport efficiently therapeutics to the posterior eye segment, minimizing drug loss and side effects. However, being the cornea the most important anatomical barrier that limits the delivery of drugs into the eye, the evaluation of permeation through this barrier is necessary. The aim of this preliminary study is to evaluate an ex vivo model useful to study the permeation of drugs and above all nanotechnology-based drug delivery systems across the cornea. This model implies the use of bovine corneas, as one of the most useful model to simulate human corneas, and Franz type diffusion cells. This should be used to evaluate the capacity of nanotechnology-based drug delivery systems to enhance and promote the entrance of drug into the eye. In particular, the use of polymeric micelles based on polysaccharide polymers is proposed. New inulin (INU) and hyaluronic acid (HA) amphiphilic derivatives (INU-EDA-RA and HA-C16) were synthetized. In addition, dexamethasone was chosen as an effective drug useful for retinal diseases. Consequently, dexamethasone loaded INU-EDA-RA and HA-C16 micelles were prepared and characterized.

MM6
Anti-inflammatory effects of formoterol and fluticasone propionate in bronchial epithelial cells

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Body: The addition of long-acting b2-agonists (LABAs) to corticosteroids improves asthma control. Cigarette smoke exposure increasing oxidative stress, increases airway inflammation and may negatively affect corticosteroid responses. The anti-inflammatory effects of formoterol (FO) and fluticasone propionate (FP) in human bronchial epithelial cells (16HBE) exposed to cigarette smoke extracts (CSE) are unknown. **Aims:** This study was aimed to explore whether FO combined with FP, counteracts some CSE-mediated effects in 16HBE including: 1) the nuclear translocation of Glucorticoid Receptor (GR); 2) the nuclear expression of NF-KB; 3) the expression of the NF-KB related cytochines IL-8 and TNFα. **Methods:** 16HBE were stimulated with CSE and/or FO and FP. Nuclear translocation of GR and NF-KB were assessed by western-blot analysis. IL-8 and TNFα exspression were valuated by Real-time PCR. **Results:** CSE decreased the expression of GR and increased the expression of nuclear NF-KB. FO combined with FP, was able to revert these phenomena in CSE stimulated 16HBE cells increasing the nuclear translocation of GR and decreasing the nuclear translocation of NF-KB. FO combined with FP reduced the expression of IL-8 and TNFα in CSE stimulated epithelial bronchial cells. **Conclusions:** The present study provides compelling evidences that FO may contribute to revert some processes induced by oxidative stress and is able to increase the anti-inflammatory effects of FP.

MM7
Different modulatory effect of the synthetic cannabinoid WIN55,212-2 on tumor cell migration

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MicroRNAs are small non-coding regulatory molecules exerting pleiotropic action in different biological processes such as proliferation, differentiation, apoptosis, migration and metastasis. Deregulation of miRNA expression has been observed in various cancers, and accumulating data suggest that miRNAs can display an oncogenic, antioncogenic or an ambiguous behavior in relationship to tumor environment. In a previous

research we showed that the synthetic cannabinoid WIN55,212-2 is able to reduce the migratory activity of osteosarcoma MG63 cells analyzed by means of wound healing assay. So we undertook a study to evaluate the biochemical mechanism through which WIN plays this action. To this purpose we evaluated the levels of miR-29b1, a member of miR-29 family which has been shown to impact critical steps in the migratory and metastatic cascade, such as EMT, apoptosis and angiogenesis. RT-PCR experiments showed that in MG63 cells 5 mM WIN increased the level of miR-29b1 of about 700-fold. This effect was accompanied by the reduction in its putative targets MMP-2, PDGF-B and N-MYC, thus indicating that the miRNA is functionally active. Moreover, cells stably overexpressing miR-29b1 did not close the wound after 48 h, mimicking the effect of WIN in untransfected control cells. Notably, ERα(+) MCF-7 and triple negative MDA-MB-231 cells, two different breast cancer models, treated with the cannabinoid migrated into the scratched area significantly faster than the respective control cells. In these cells WIN also increased the level of miR-29b1 targets. Therefore, differently from osteosarcoma cells, these preliminary observations seem to indicate that WIN promotes migration ability in breast cancer cells. The reasons for this diverse behaviour could rely on miR-29b1, whose expression can change in different cell types or show temporal differences dictated by cell physiology and tumor microenvironment impact. Studies are in progress to shed light on the molecular mechanisms underlying this different response.

MM8
A study of cell-cell fusion through generation and culture of osteoclasts from RAW 264.7 cells

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One of the unique ability of macrophages is to fuse each other to form multinucleate giant cells (MGCs). The most characterized type of MGCs are the osteoclasts (OCs). Two osteoblast-derived cytokines, M-CSF and RANKL, control osteoclastogenesis through engagement of their cognate receptors c-fms and RANK, respectively. RAW264.7 macrophages are considered pre-OCs and become OCs within 4-5 days after stimulation with RANKL. They respond recruiting TRAF6, a receptor-associated factor, which triggers a cascade of transcription factors, including NF-□B, NFATc1and c-Fos. These factors switch on the expression of OC-specific genes, such as those coding for the enzymes TRAP and cathepsin K, and the fusion-specific molecules DC-STAMP and ATP6v0d2. Ultimately, pre-OCs TRAP+ fuse to form multinucleated mature OCs TRAP+. Dynamic reorganization of the cytoskeleton mediates cell fusion, through drastic and regular variations of filopodia and podosomes. In this study, by means of IF, we found that all mononuclear cells showed podosomes 1 day after RANKL stimulation. At the same time, RANKL treatment inhibited phagocytic ability of RAW 264.7 cells dose-dependently, suggesting a decrease of macrophages and a gain of pre-OCs properties. After 3 days from RANKL addition, RAW264.7 cells started to form cell aggregates. The number and density of podosomes, which appeared as dots on pre-OCs, increased during OC maturation. Some cells in the aggregates possessed podosomes assembled into small actin rings, which eventually formed podosome belts on the 4th day. Multinucleated TRAP+ cells, i.e. exhibiting TRAP activity as intense cytoplasmic red staining under light microscopy, were considered as differentiated OCs. Electron microscopy images showed 4-days treated cells at various steps of cell fusion. Canonical NF-□B pathway was induced rapidly in pre-OCs in response to RANKL and, by means of IF; we found that a second peak of induction for NF-κB signaling is required for terminal differentiation of pre-OCs.

MM9

Integrated computational and experimental approaches for the identification of new molecules with readthrough activity on premature termination codons (PTCs) in cystic fibrosis cells

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Cystic Fibrosis (CF) patients with nonsense-mutation in the CFTR (Cystic fibrosis transmembrane conductance regulator) gene generally make virtually no CFTR protein and thus often have a more severe form of CF. Recently, Ataluren (PTC124; Translarna) was suggested to induce the readthrough of premature termination codons mainly the UGA codon. However, despite promising results there is not a general consensus on efficacy and mechanism of action. The design of new small molecules (PTC124 related) together with the understanding of their mechanism of action could lead to new pharmacologic approaches for the cure of CF. This work was aimed to identify new molecules (PTC124 analogues) with readthrough activity and to evaluate their efficacy in CF cells. In particular, the experiments were conducted in different cell model systems: 1- human cells transfected with vectors containing PTCs in reporter genes; 2- primary human bronchial epithelial cells 3- immortalized epithelial cells of cystic fibrosis (CF) patients. Design and synthesis of the new PTC's read-through promoters was based on the results obtained by a virtual screening approach. We synthesized 18 analogues of the PTC124 and tested some of them in three different biological models. The FLuc assay and IB3.1 cell lines were used to test the new identified products. Three of these new compounds showed high read-through capacity in CF cells. Finally, computational studies were aimed to model the interaction between the bioactive synthesized compounds and the possible cellular target, in order to understand the mechanism of action of the new synthesized analogues.

MM10

Maternal high fat diet consumption during pregnancy and lactation: impact on intestinal morphology and function in preweaning offspring

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Different evidence supports an important role for maternal obesity in the development of childhood obesity and subsequent adult disease. This study is addressed to investigate if and to which extend maternal high fat feeding would induce compensatory and adaptative responses in gut predisposing to the eventual development of paediatric obesity. Adult female mice were divided into two groups fed with i) high fat (HF) diet and ii) standard chow (SC)diet, during pregnancy and lactation. HF mothers showed a significant weight gain, higher levels of blood glucose and an abnormal glucose tolerance compared to SC mother, indicating the establishment of metabolic syndrome. Then, offspring subdivided according to maternal diet, O-SC pups birthed from mothers fed SC, whereas O-HF pups birthed from mothers fed HF diet, and morphological and functional experiments were performed in the small intestine 2 developmental ages, early suckling (P2) and late suckling (P15) to evaluate the contribute of maternal milk in the development of obesity. O-HF at P2 and P15 did not show significant changes in the morphology of the small intestinal wall (villus height, depth of the crypt, villus width near the crypt and thickness of the muscular layer) compared to O-SC. Moreover in agreement with morphological data, no difference has been found in the amplitude and frequency of the intestinal spontaneous mechanical activity from O-HF compared to O-SC. The contractile and relaxant responses to well know drugs as the muscarinic receptor agonist, carbachol, and the □-adrenergic receptor agonist, isoproterenol, were similar in both groups of animal. This study suggested that during lactation,

maternal high fat feeding did not induce any compensatory and adaptative responses in gut that could suggest a predisposition to the development of pediatric obesity. Further experiments at later ages are currently in progress.

MM11

Expression of non-cholinergic system components in the airways epithelium of COPD patients

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Background: Acetylcholine (ACh) is synthesized by Choline Acetyl-Transferase (ChAT) that exerts its physiological effects in airway epithelial cells via muscarinic receptors activation, particularly mAChRM3 and acts as an intracellular autocrine modulator. **Aims:** We aimed to evaluate ChAT, ACh and mAChRM3 proteins expression a) *"in vitro":* in human epithelial cell lines 16HBE and A549 stimulated with cigarette smoke extract (CSE); and b) *"ex vivo":* in surgical specimen samples from central and distal airways of healthy control (HC), healthy smoker (HS) and COPD. **Materials and Methods:** We investigated ChAT, ACh and mAChRM3 proteins expression in 16HBE and A549 CSE-treated cells by flow cytometry and immunofluorescence, and in the epithelium from surgical specimens from central and distal airways of HC, HS and COPD patients, by immunohistochemistry. Furthermore, to evaluate the involvement of Non-neuronal Cholinergic components in pathological conditions of COPD, we evaluated the expression of these markers in metaplastic areas of epithelium. **Results:** *In vitro*, mAChRM3, ChAT and ACh expression was significantly increased in 16HBE stimulated with CSE but not in A549. Accordingly CSE increased ChAT/mAChRM3 and ChAT/ACh co-localization in 16HBE but not in A549. In *ex-vivo* study, ChAT and ACh expression were higher than in HC subjects in central airways of HS and COPD the mAChRM3. Furthermore, mAChRM3 expression was higher in COPD and HS than in HC only in distal airways. Finally, the most of COPD subjects had high score of mAChRM3, ChAT and ACh expression expression, in metaplastic areas. These findings might suggest that these markers are involve in the mechanisms of pathological conditions in the airways of COPD. **Conclusion:** An altered expression of non-cholinergic system components might are generated by cigarette smoke habit in the airway epithelial cells promoting the mechanisms of proliferation involved in the pathogenesis of COPD.

MM12

In silico insights and molecular characterization between IKK kinase and some ligands

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Protein kinases family plays pivotal roles in nearly every aspect of cellular function. They control metabolism, transcription, cell division and programmed cell death through modification of the protein target function. Typically, the reaction catalyzed by protein kinase is $MgATP^{1-}$ + protein–O: H → protein–O: $PO3^{2-}$ + MgADP + H^{+}. Protein kinases family has become one of the most important drug targets over the past two decades. In particular, most of the FDA-approved protein kinase inhibitors are competitive with respect to ATP. IKKα/β is a member of the protein kinase family and has a central role in NF-κB activation in response to different pro-inflammatory stimuli, since the active form of the enzyme leads to IkBα/β phosphorylation and ultimately to the activation of NF-κB. Our data suggest that ferulic acid (FA) may interfere with IKK/IκB/NF-κB signalling, both reducing NF-κB nuclear translocation and the

phosphorylation of IKKα/β and IkBα in LPS-induced Raw 264.7 cells. The regulatory mechanisms governing this phosphorylation event are not well understood. It is known that the molecular mechanism underlying the inhibition of kinase proteins consists of the formation of a ligand-receptor complex.

In support, of these experimental outcomes we carried out computational studies by means of Molecular Docking and Induced Fit Docking (IFD) protocols, in order to get new insights at molecular level on the interaction between FA and the human IKK-β (PDB: 4KIK). Furthermore, to better understand the binding mode and the molecular pose, we selected some IKK-β known ligands such as Staurosporine and BMS 345541, both known as potent ATPase competitors, and also ATP. These studies permitted us to get the preliminary binding affinity on IKK-β of all the analysed ligands.

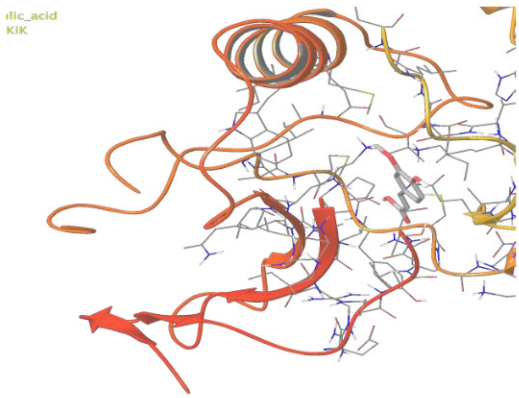

Figure 1. Ferulic acid docked in the Ikk-beta ATP site.

MM13
A rapid method for the quantification of caffeine and chlorogenic acids in coffee

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Coffee is an extremely popular beverage daily consumed. It has been for decades the most commercialized food product and the most widely consumed beverage in the world. It is noteworthy that today coffee is considered as a functional food, primarily due to its high content of compounds that exert invigorating and antioxidant effects as: caffeine and chlorogenic acids. Caffeine is a secondary metabolite of coffee plants and it is an alkaloid acting as a mild psychoactive stimulant drug on the central nervous system. Chlorogenic acids (CGAs) are phenolic compounds deriving by esterification of trans-cinnamic acids with quinic acid. In the last years, these compounds have received much attention for their beneficial effects against neuro-degeneration and aging. However, positive or negative effects on health and final quality of the coffee are dependent on the concentrations of these molecules. Thus, due to the increasing heed of consumer to the food safe and to effect on quality of the coffee products there is a growing interest in the monitoring and quantification of caffeine and CGAs in coffee. In this research work, a simple method for the simultaneous determination of caffeine and CGAs content in green coffee was reported. The method was based on the use of UV/Vis absorption. It is relevant that the quantification of both caffeine and chlorogenic acids was performed without their preliminary chemical separation despite the spectral overlap in the range 250-350 nm. Quantitative determination was obtained analytically through deconvolution of the absorption spectrum of ethanolic extract obtained by coffee. The bands used to make the deconvolution were the absorption bands of the caffeine and the chlorogenic acid standards. The molar extinction coefficients in ethanol solution at 70% were $\varepsilon_{(272\ nm)}$ =12159(M cm)$^{-1}$ for caffeine and $\varepsilon_{(330\ nm)}$=27025(M cm)$^{-1}$ for chlorogenic acid. The estimate of the concentration was in agreement with those obtained by HPLC quantification. The described method is fast and simple, allowing to realize routine controls during coffee production, it could be applied on green, roasted, and espresso coffee.

MM14

On the direct and cell-mediated interactions between Metformin and beta-amyloid peptide

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Several studies show that individuals with Type2 diabetes (T2DM) or Obesity have a two-fold greatest risk of developing Alzheimer's disease (AD). AD is the most common cause of dementia in the elderly. Pathohistological hallmarks of AD are soluble β-amyloid (Aβ) oligomers or insoluble plaques or neurofibrillary tangles. A possible link between AD and T2DM /obesity could be due to long-term use of antidiabetic drugs. Some evidences indicate that metformin, the usually recommended insulin-sensitizing drug, increases the production and aggregation of Aβ. In this study we utilize a mouse model to investigate the ability of metformin to reach the brain by imaging studies and biochemical analysis. Metformin was administered for 3 months and an increase of the level of expression of proteins involved in AD neurodegeneration was detected. Immunofluorescence analysis with anti-Aβ antibodies and Th-T staining revealed presence of amyloid plaques. In order to understand whether metformin is also able to directly interact with Aβ, we performed in vitro extrinsic fluorescence, dynamic light scattering (DLS) circular dichroism (CD) and Atomic Force Microscopy (AFM) experiments, by incubating the amyloid peptide with and without metformin. We found that metformin increases the lag time and reduces the extent of fibrillation (ThT plateau level). From DLS experiments, these species result lower in hydrodynamic size. In addition, the typical conversion to beta structure that typically accompanies the fibril formation is slowed down by the presence of metformin. Finally, AFM measurements confirm that the presence of metformin sizably reduces the formation of large amyloid fibers, probably favoring the smaller aggregates. In conclusion, *in vitro* metformin inhibits fibrillogenesis stabilizing small oligomeric species that probably have high toxicity potential that could reflect the physiological features observed *in vivo*.

MM15

Social closeness, salivary hormones and physical exercises

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Introduction: Saliva collection and analysis is quickly becoming a useful and non-invasive tool for the evaluation of sport biomarkers. The aim of this study is to create a multidisciplinary assessment model, which can help to provide psychological and physiological responses, related to sport performances, social closeness and performance anxiety management in team sports. **Materials and methods**: We enrolled in our research 26 female volleyball players aged 13 ± 1 years old of three different teams (T_1: 12 players; T_2: 9 players; T_3: 5 players). Saliva collection was carried out before and after the match for every team. Then we analyzed cortisol and progesterone concentrations through Elisa standard kits. **Results**: The results of the T-test performed on the total results showed a statistically significant relationship ($p < 0.05$) in cortisol levels pre and post match: in fact, it has been shown a statistical significant decrease ($p < 0.001$). The analysis performed using just samples post match shows a negative correlation between social closeness, cortisol and progesterone levels, with $p < 0.010$ for progesterone vs social closeness and $p < 0.012$ for cortisol vs social

closeness, which indicates that increasing of one of the two hormones reduces relationship. About the winner teams and the looser teams, there is a negative correlation between pre match cortisol levels and performance anxiety ($p < 0.042$).

MM16
p65(-1), a new isoform of NF-kB complex and its role in inflammation.

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Acute inflammation is a physiological response devised to eliminate the source of damage and to restore homeostasis tissue. However, when this mechanism is deregulated, if it becomes chronic it can even promote malignant cell transformation and carcinogenesis. The transcription factor NF-kB comprises a family of five members, characterized by the presence of a highly conserved amino-terminal *Rel Homology Domain* (RHD), that plays a critical role in inflammatory and immune responses. This domain is responsible for dimerization, nuclear localization and interaction with IkBs inhibitor. All the members can form homo- or heterodimers, but the dimer most represented is p65/p50. Glucocorticoid based therapy is still the most commonly used treatment for chronic and acute inflammation. Commonly, glucocorticoids bind the ligand-binding domain of glucocorticoid receptor (GR) to promote nuclear translocation and transcriptional activation of anti-inflammatory genes. The GR activated transcription factor also physical interact with the p65 subunit of NF-kB complex to repress inflammation. In our lab, we discovered both in mice and humans, a new isoform of p65 subunit called p65(-1). Transcriptional assays demonstrated that this protein acts oppositely to the *wild type* one, because it can increase GR response after glucocorticoid treatment. We hypothesize that this new isoform might be used as a drug able to increase the anti-inflammatory response induced by glucocorticoids. As a consequence, the glucocorticoid dose might be reduced, thus limiting the side effects related to their use. We demonstrated that the heterodimer p65/p65(-1) is able to activate transcription by NF-kB response elements, more than p65/p50. The p65 dimer interface is formed by 13 aminoacids, but the main responsible for interaction with p50 are Phenilalanine 213 (Phe 213), Leucine 215 (Leu 215) and Histidine 245 (His 245). We have performed site-direct mutagenesis in this specific region, in order to study if an amino acid substitution can compromise the ability to form a heterodimer, while maintaining the ability to induce an anti-inflammatory response.

MM17
Role of renin-angiotensin system in colonic dysmotility associated with bowel inflammation in rats

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Dysregulation of different mediator systems could contribute to the gut dismotility in inflammatory bowel diseases (IBDs), chronic disorders characterized by an exasperated immune response disturbing gut functions. Among these, Angiotensin II (Ang II), the main peptide of renin-angiotensin system (RAS), can participate in inflammatory responses and RAS components are increased in IBD patients. Since RAS has emerged as gut motility regulator, our objectives was to investigate, in an IBD rat model, the RAS functionality and its eventual contribution to colonic dismotility. Experimental colitis was induced in rats by intracolonic administration of 2,4-dinitrobenzenesulfonic acid (DNBS). Drug effects on the longitudinal colonic muscular contractility of normal and DNBS-treated rats were characterised in vitro, using organ bath-technique. Colonic preparations from DNBS rats, showed a low in amplitude spontaneous activity and a depressed responsiveness to pharmacological agents, such as the cholinergic agonist, carbachol and the β-adrenergic receptor agonist isoproterenol, compared to normal rats. Ang II induced muscular contraction in

normal and inflammatory conditions, whose amplitude was decreased in DNBS rats. In both preparations the AT1 receptor antagonist, losartan, reduced Ang II effects. The AT2 receptor antagonist, PD123319, ineffective in control rats, enhanced Ang II contractile responses in DNBS rats. The neural toxin, TTX and L-NNA, NO synthase inhibitor, were ineffective in control rats, but increased contractile response in DNBS rats. The joint application of PD123319 and TTX or L-NNA did not have additive effects. Interestingly, PD123319 improved the spontaneous activity and carbachol and isoproterenol responses in DNBS animals. In conclusion, Ang II contracts rat colonic longitudinal muscle via post-junctional AT1 receptors. Under bowel inflammation AT2 receptor recruitment, likely on inhibitory nitrergic neurons, decreases colonic contractility and would counteract AT1 activity. Pharmacological manipulation of RAS system could be considered in the attempt to improve the treatment of intestinal dismotility in IBDs.

MM18

Pharmacological characterization of dopamine effects on the mechanical activity of longitudinal and circular muscles in human colon

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Dopaminergic neurons are present in both enteric plexuses in different animal species and D1- and D2-like receptors are widely expressed throughout the gut suggesting a role for dopamine (DA) as modulator of gastrointestinal (GI) functions, as motility. So far, it is know that pathological alterations of enteric dopaminergic system may be implicated in some GI motor disorders, as dyspepsia and gastroparesis, but no clear information is available about dopamine effect in the human gut. The aims of this study were to characterize DA effects on human colon contractility, the receptor subtypes involved and the possible differences in the dopaminergic receptor activation between longitudinal and circular muscle. Mechanical responses to DA and dopaminergic drugs were examined *in vitro* as changes in isometric tension in circular and longitudinal muscle strips from human colon. DA (1-300 μM) induced a concentration-dependent contraction of circular muscle strips, reduced by SCH 23390, D1-like receptor antagonist, and mimicked by SKF 38393, D1-like receptor agonist. D2-like receptor agents were ineffective. D1-like mediated contraction was unaffected by the neural blocker TTX or by the muscarinic antagonist atropine. In contrast, in the longitudinal muscular strips DA caused a concentration-dependent relaxation, antagonized by domperidone, D2-like receptor antagonist, and mimicked by bromocriptine, D2-like receptor agonist. D1-like receptor agents were ineffective. The D2-like mediated relaxation was TTX-insensitive and unaffected by L-NNA, NO synthase inhibitor, but partially reduced by propanolol and SR59230A, □2 and □3-adrenergic receptor antagonist respectively. In conclusion, DA in human colon induces opposite effects activating different classes of dopaminergic receptors. DA contracts the circular muscle layers via excitatory postjunctional D1-like receptors. Indeed, DA relaxes longitudinal muscle activating inhibitory D2-like receptors and in part □2 and □3 adrenergic receptors. This opposite effects on the muscular layers, may indicate a possible contribute of dopaminergic receptor activation in the modulation of peristaltic reflex.

Oncologia (ON)

ON1

The small molecule HSP90 inhibitor luminespib (AUY922) for treatment of hepatocellular carcinoma

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HCC (Hepatocellular Carcinoma, HCC) is a highly malignant tumor. The traditional surgery, chemotherapy and radiation therapy increased the 5-year survival in patients with HCC only by 6%. Therefore, there is the need to develop new therapeutic strategies. The HSP90 (heat shock protein 90) is one of the most important molecular chaperones and is highly expressed in different types of tumors. However, the role of HSP90 in hepatocarcinogenesis remains unclear. In this study, we analyzed HSP90 expression in primary human HCC tissues and evaluated the antitumor effects of a specific inhibitor of HSP90, luminespib (AUY922), a synthetic molecule, in different human HCC cell lines. We found that HSP90 expression was significantly higher in HCC tissues compared to that of peritumoral liver chirrhotic tissues ($p< 0.001$), suggesting that the presence of HSP90 might be involved in hepatocarcinogenesis. We therefore investigated the antitumor effects on cell viability of luminespib in HCC cell lines. The treatment with luminespib reduced the viability of HCC cells, HepG2, Huh7, Hep3B, PLC/PRF/5 and SNU475, in a time- and dose-dependent manner. In addition, treatment led to the simultaneous inhibition of the expression of some crucial factors known to be involved in hepatocarcinogenesis, such as protein kinase B (AKT), extracellular signal-regulated kinase 1/2 (ERK1/2), and epidermal growth factor receptor (EGFR), and fragmentation of β-catenin, in a caspase-dependent manner, resulting in the inhibition of β-catenin-mediated transcriptional activity. The inhibition of HSP90 triggers apoptosis and limited cell migration ability of HCC cell line SNU475. In addition, combination of luminespib with sorafenib synergistically inhibited cell viability of HCC cells. In conclusion, HSP90 is a promising therapeutic target in HCC and luminespib could be a drug candidate for the treatment of HCC.

ON2

Delivery of shNupr1 plasmid by solid lipid nanoparticles reduces the expression of *Nupr1* gene in hepatocellular carcinoma cells.

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Hepatocellular carcinoma (HCC) is one of the most frequent cancers worldwide. Effective therapy to this cancer is currently lacking, creating an urgent need for new therapeutic strategies. Gene therapy approach, that implies any procedure intended to treat a disease by genetically modifying the cell of a patient (transferring genes, gene segments, or oligonucleotides), may provide a promising strategy.
To obtain therapeutic effects, nucleic acids need to cross several biological barriers and be protected from the degradation by nucleases, gaining access to their intracellular targets. Therefore, it is necessary to use biocompatible carriers to facilitate their translocation across the cell membranes protecting them from being degraded while circulating in the bloodstream. At this purpose, cationic solid lipid nanoparticles (cSLN),

able to bind nucleic acids by electrostatic interactions, have emerged as promising vectors due to their versatility and low toxicity. Nupr1 is a small multifunctional protein whose expression is induced by several stresses. It interacts with numerous partners to regulate cell cycle, programmed cell death, autophagy, chromatin accessibility and transcription. For all these reasons *Nupr1* might be a protein whose blockade would prevent cancer progression and metastasis development. In the present study, we aimed to develop cSLN able to efficiently bind, protect and deliver shNupr1 plasmid for the treatment of hepatocellular carcinoma. The cSLN were prepared, characterized in terms of size, polydispersity index and zeta potential, and complexed with shNupr1 plasmid, in presence or absence of trehalose, at different weight ratios. The physical binding between SLN and the nucleic acids was confirmed by zeta potential measurements and electrophoretic mobility studies. Finally, *in vitro* biological assays confirmed that these nanosystems were not cytotoxic and efficiently knockdown *Nupr1* expression in Hep3B cells. The obtained data suggest that these nanosystems may be useful for in vivo applications as nonviral vectors for the treatment of HCC.

ON3

Crude extracts of *anemonia viridis* affect the growth and viability of selected tumour cell lines

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It is known that most of the available cancer treatments cause severe side effects caused by their non-selective cytotoxicity. Another emerging problem regarding chemotherapy is cancer drug resistance. Therefore, the search for novel chemotherapeutic agents with anti-proliferative activity remains an important target for scientists. In the last few years, marine species have been investigated for the presence of natural products with anticancer activity. Among marine invertebrates, cnidarians are one of the most interesting biological systems related to the isolation and production of bio-active molecules. In particular we have focused our attention on *Anemonia viridis*, a widespread Mediterranean species. Using a solid phase Sep-Pak C8 column, low molecular weight proteins were fractionated from the body of the sea anemone *Anemonia viridis.* Using acetonitrile (ACN)/water solutions, four different extracts (15%, 30%, 45% and 60%) were evaluated for their cytotoxic activity by means of erythrocyte haemolysis, MTS and LDH assays. Finally, the antiproliferative activity of three of these fractions were studied on PC3, PLC/PRF/5 and A375 human cancer cell lines. Our analysis showed that the four ACN fractions showed different protein contents and diverse patterns of reactivity towards human PBMC and cancer cell lines. Cytotoxicity assays showed that the 45% and 60% ACN fractions had a toxic effect on human cells. On the other hand, whereas the 15% and 30% ACN fractions displayed a very low toxic effect, they instead had an antiproliferative effect on cancer cell lines. Our study reports the evaluation of the cytotoxic and antiproliferative activities of four low molecular weight protein fractions extracted from the body of *Anemonia viridis*, opening the way to future characterisation of natural products for anticancer therapies.

ON4

S100 proteins in breast cancer: multiomics-based analyses

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S100 gene family is the largest subfamily of calcium binding proteins, expressed in tissue and cell-specific manner. Within cells, S100 have been involved in the regulation of proliferation, differentiation, apoptosis, energy metabolism, inflammation, migration and invasion. Extracellular S100 proteins act in an autocrine and paracrine manner and regulate cell proliferation, differentiation, survival and migration. S100 proteins

play important roles in the development and progression of tumors due to their multifunctional roles. However, the occurrence, the role and the possible coordination of this group of proteins in breast cancer is still poorly known. We previously describe a large-scale proteomic investigation performed on breast cancer patients for the screening of multiple forms of S100 proteins[1,2]. Our results have shown that the majority of S100 proteins are preferentially expressed in the tumor mass compared with the normal adjacent tissue and that some S100 protein members were ubiquitously expressed in almost all patients, while others appeared more sporadic among the same group of patients. More interestingly, patients which developed distant metastases showed a general tendency of higher S100 protein expression, compared to the disease-free group. Present study was aimed to assess the gene expression levels of the S100 protein family members utilizing a breast cancer dataset generated on Affymetrix microarrays technologies[3]. GOBO (Gene expression-based Outcome for Breast cancer Online) is a user-friendly online tool that allows, also, the identification of co-expressed genes and association with outcome in an 1881 breast cancer samples. Other important association with breast cancer outome was carried out by Kaplan Meir-plotter database[4]. Integrating results obtained by proteomic and trascriptomic analysis of S100 proteins highlight their important involvement in breast cancer progression. Future studies are needed to disclose molecular mechanisms and signaling pathways that define the multiple and specific roles of S100 proteins in breast cancer.

[1] Cancemi P *et al*. BMC Cancer 2010, 10:476.
[2] Cancemi P *et al*. Proteomics Clin Appl 2012, 6:364-73
[3] Ringnér M *et al*. PLoS One 2011, 6:e17911.
[4] Gyorffy B *et al*. PLoS One 2013, 8:e82241.

ON5

Construction and validation of a retroviral vector for the inducible expression of the p14^{ARF} tumor suppressor gene in human cells

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Aneuploidy is a common feature of human tumor cells. Currently, is still debated if aneuploidy is a cause or consequence of cancer. The p14^{ARF} tumor suppressor gene is mutated in several cancers and it has been suggested its involvement in the maintenance of chromosomal stability in MEFs via p53-dependent and p53-independent mechanisms. We engineered a retroviral vector for the inducible expression of p14^{ARF} in nearly diploid HCT116 tumor cells. To this aim the p14^{ARF} cDNA has been cloned into a tetracycline-regulated retroviral vector (pBPSTR1). The recombinant retroviruses were produced in the Phoenix packaging cell lines and used to infect HCT116 cells, then we selected a cellular population of HCT116 cells that stably re-expressed p14^{ARF}. Initially, we evaluated the efficiency of the retroviral infection and the correct functioning of the Tet-Off system by using the retroviral vector pBPSTR1-H2B-GFP to infect HCT116 cells. In the presence of 100 ng/ml of Doxycycline for 48h, the HCT116 $^{pBPSTR1\text{-}H2B\text{-}GFP}$ cells showed a remarkable decline in the number of H2B-GFP positive cells. Conversely, the number of H2B-GFP positive cells increased when the Doxycycline was removed from the culture medium confirming the inducibility of the system. Initial characterization of HCT116 cells re-expressing p14^{ARF} showed no ploidy changes and normal cell proliferation. This work represents a preliminary step for subsequent studies on the role of p14^{ARF} to counteract aneuploidy.

ON6
Anti-proliferative and pro-apoptotic effects of oleuropein and hydroxytyrosol in melanoma cells

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Metastatic melanoma is the most deadly form of skin cancer that arises from transformed melanocytes of the basal region of epidermis. Melanoma cells are resistant to a wide range of chemotherapeutic and radiotherapeutic treatments, therefore, there is an urgent need for new and efficient therapeutic agents. In this contest, we studied the olive oil components that have been shown to induce the death for apoptosis of several cancer cells mainly through its phenolic compounds. The three phenolic compounds in highest concentration in olive oil are the glycoside oleuropein, hydroxytyrosol (3,4-dihydroxyphenyl ethanol) and tyrosol. In this study we investigated the anti-proliferative and pro-apoptotic potentials of oleuropein, tyrosol and hydroxytyrosol in A375, HT144 and M74 human melanoma cell lines using proliferation assays (MTS assay), western blot and DeadEnd™ Colorimetric TUNEL assay. In particular, the MTS assays showed that 200 μg of oleuropein and 250 μg of hydroxytyrosol remarkably reduced cell viability of treated melanoma cells. However tyrosol does not affect melanoma cell viability. Moreover, the results reported suggest that oleuropein and hydroxytyrosol treatments induces the melanoma cells apoptosis through PARP inactivation, caspase 8 and caspase 9 activation as well as increasing expression of p53 and gH2AX. Furthermore oleuropein and hydroxytyrosol cell apoptosis was confirmed through TUNEL experiments. In conclusion, these data show that the main phenolic compounds of olive oil, hydroxytyrosol and oleuropein, have potential chemotherapeutic properties and might provide basis for the design of new therapeutic agents for effective treatment of this highly invasive tumour.

ON7
Implication of runt-related transcription factor 2 (RUNX2) in controlling cell growth and sorafenib resistance in human hepatocellular carcinoma cells

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Many reports have shown that runt-related transcription factor 2 (RUNX2) has an oncogenic role in different human malignancies, while its role in HCC still remains unclear. In our previous studies, we found that the *RUNX2* gene was down-regulated in HCC cells upon silencing of *nuclear protein 1* (NUPR1) gene, a new target in HCC. Intriguingly, recently in ovarian cancer cells it has been demonstrated that *RUNX2* gene silencing provides a strong down-regulation of *NUPR1* expression suggesting the existence of an interaction between these two transcription factors. In the present study, we examined RUNX2 mRNA expression in HCC tissues compared to normal liver tissues. Real-time PCR analysis showed that RUNX2 mRNA was over-expressed in 8 out of 12 (66.6%) HCC tissues, suggesting that *RUNX2*, as well as *NUPR1*, may have an important role in hepatocarcinogenesis. Therefore, to ascertain the functional role of RUNX2 in HCC, we studied the biological effects on regulation of cell growth, migration and chemoresistance upon *RUNX2* gene silencing in HCC cells using specific human siRNA. *RUNX2* gene silencing significantly decreased HCC cell growth and migration and increased cell sensitivity to sorafenib treatment. Interestingly, in accordance with data reported by others, gene expression analysis showed that *RUNX2* suppression strongly down-regulates *NUPR1*, as well as *NUPR1* downstream target genes *RelB* and *IER3*. These data strongly suggest the existence of a *NUPR1/RelB/IER3/RUNX2* pathway that may act as an auto-regulatory loop. The

identification of the NUPR1/RELB/IER3/RUNX2 pathway as a potential therapeutic target may contribute to the development of new treatment strategies for HCC management.

ON8
A biclustering approach for the analysis of miRNA expression profiles

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RNA sequencing (RNA-seq) is a New Generation Sequencing (NGS) method used for the analysis of transcripts and differential gene expression profiles. MicroRNA (miRNAs, 22--25 nt long) are, among small non coding RNAs (sncRNA) obtained through RNA-seq, key regulators in multiple cellular functions, as they play a crucial role in different physiological processes. miRNAs are in fact differentially expressed in several types of cancer, in specific tissues and during specific cell status. Clustering algorithms have been applied to microarray data in order to discover groups of genes (clusters) that are co-regulated with respect to certain experimental conditions. Because many regulation mechanisms involve only set of genes and limited set of experimental conditions, a new approach is needed. In this context, biclustering techniques represent suitable approaches because they allow to separate, in a data matrix, groups of rows and columns, standing for genes and samples , that exhibits similar values or similar characteristics. We present a biclustering approach in order to identify some patterns of miRNA expression deregulation in human breast cancer versus healthy controls. We applied the Iterative Signature Algorithm (ISA) tool, which has proved one of the most efficient when applied to gene expression datasets. Considering a real word breast cancer dataset, composed of 185 samples, we identified 12 miRNA biclusters, each of them involving different types of tumor samples and miRNA families. We showed the association between specific sub-class of tumor samples having the same immuno-histo-chemical (IHC) and/or histological features. Biclusters have been validated in the current scientific using the MetaMirClust and UCSC Genome Browser online tools, as well as another biclustering algorithm (SAMBA). The proposed biclustering led to the identification of different groups of miRNAs and patient conditions, that eventually have to be validated by in-vitro experiment.

ON9
Possible regulatory mechanisms responsible for the high expression of serpin protease inhibitor PI-9 in ER+-derived breast cancer stem cells

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Breast cancer (BC) is the most common endocrine cancer and the second leading cause of cancer-related death in women. About 75% of BCs expresses high levels of estrogen receptors that sustain the tumor growth. As a consequence, cancer cells acquire the ability to escape immune surveillance's signaling. Although some studies explored the role of PI-9 in BC cells, its presence has not been investigated in cancer stem cells so far. In this research, tertiary tumorspheres were obtained from estrogen receptor-α positive (ERα+) BC MCF7 cells and studies were performed to evaluate their stem identity. These tumorspheres showed high levels of stemness markers (Nanog, Oct3/4 and Sox2) and self-renewal ability. The exposure to estrogens (17-β estradiol and genistein) increased the number and sizes of tumorspheres as well as the level of the proliferating cell nuclear antigen (PCNA). The analysis of the three isoforms (66, 46 and 36 kDa) of ERα disclosed that tertiary tumorspheres exhibit a marked increase in ERα36, while the level of ERα66, which is highly expressed in MCF7 cells, dropped. Then, we analyzed the granzyme B inhibitor PI-9, which is transcriptionally regulated by ERα66. Surprisingly, we found that tertiary tumorspheres express a higher

level of both PI-9 protein and mRNA than MCF7 cells, despite the reduced level of ERα66. The high content of PI-9 might be ascribed to the activation of proliferative CXCR4/phospho-p38 axis which was observed only in tertiary tumorspheres. Taken together, these events could supply a selective advantage to BC stem cells by interfering with immune surveillance systems and open the way to new possible targets for BC treatment.

ON10
A new pH responsive polymer based on inulin for siRNA Delivery

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siRNA-based therapeutics hold great potential for treatment of cancer by targeting signalling pathways that promote tumor progression. However, many challenges, including rapid nuclease degradation and poor cellular uptake, need to be addressed in order to carry these molecules into clinical trials. The goal of the work was that to produce an inulin derivative, Inulin-g-imidazole-g-diethylenetriamine (INU-IMI-DETA), bearing diethylentriamine chains (DETA) and imidazole groups (IMI) with good potential as polymeric vector for siRNA. This because DETA and IMI groups are able respectively to give strong polycation properties to resulting copolymer and to improve endosomal escaping exploiting the proton sponge effect. Moreover, these polymer derivatives bearing diaminoethane side chains exhibit a peculiar two-step protonation behavior that facilitates membrane destabilization at the acidic pH of late endo-lysosome. The experimental results showed that INU-IMI-DETA exhibited strong cationic characteristics, high solubility in the pH range 3-5, self aggregation triggered by pH increase and physiological salt concentration as well as an high buffering capacity in the endosomal pH range of 7.4-5.1. INU-IMI-DETA was tested as siRNA complexing and delivering agent and a specific two-step procedure was followed to obtain stable INU-IMI-DETA complex nanoaggregates (ICONs) into DPBS pH 7.4. This lead to produce siRNA loaded nanoparticles with minimized surface charge and suitable size for parenteral administration. In vitro studies on breast cancer cells, expressing luciferase gene, demonstrated that ICONs had no cytotoxic effect in a wide range of concentration and that are able to produce a satisfactory luciferase knockdown. Moreover, Bafilomycin A1 inhibited transfection, indicating that the copolymer favors the system escape from endolysosomal compartment.

ON11
Melanoma cells release extracellular vesicles which contain H1° RNA-binding proteins

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G26/24 oligodendroglioma cells produce EVs that contain pro-apoptotic proteins, such as FasL and TRAIL, able to induce neuronal- [1] and astrocytic- [2] death. Cancer cells release EVs [3] through which transferring proteins, such as extracellular matrix remodelling proteases [4], and H1°, a differentiation-specific histone [5]. By releasing H1°, cells could escape differentiation cues [5]. To verify the role of EVs in releasing specific proteins and mRNAs, in this study we used A375 melanoma cells. EVs were purified from cell culture media as previously reported [1, 2]. T1 RNase-protection assays were performed on total cell lysates and EVs, as described elsewhere [6]. RNA-binding proteins (RBPs) were isolated by using a biotinylated H1° RNA as a bait [7]. Melanoma cells were found to synthesize H1° and secrete it via EVs. Moreover, EVs also contain H1° mRNA. Interestingly, H1° histone sorted to vesicles seems to be sumoylated. By T1 RNase-protection assay, we evidenced in EVs three main H1° RNA-protein complexes, the most abundant of which has a molecular mass of around 65 kDa. By using as a bait biotinylated H1° RNA, we isolated a few proteins, then analyzed by mass spectrometry. The most abundant protein was

myelin expression factor-2 (MYEF2), which has a molecular mass of about 60 kDa. Finally, we confirmed MYEF2 presence in EVs by western blot.
[1] D'Agostino et al. 2006, Int J Oncol 29:1075-85.
[2] Lo Cicero A et al. 2011, Int J Oncol 39:1353-7
[3] Di Liegro et al. 2015, Biomed Res Int, 2015, Article ID 152926.
[4] Lo Cicero A et al. 2012, Matrix Biol 31:229-33
[5] Schiera G et al. 2013, Int J Oncol 43:1771-6
[6] Scaturro et al. 1998, J Biol Chem 273:22788-91
[7] Scaturro et al. 2003, Int J Mol Med 11:509-511

ON12
CENPE depletion induces aneuploidy that is reduced by the tumor suppressor p14 ARF

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The Spindle Assembly Checkpoint (SAC) is an intracellular mechanism acting to maintain genome stability during mitosis. Reduced expression of its components induces chromosome segregation errors and aneuploidy. Centromere Protein E (CENP-E) has an essential role in the SAC by allowing kinetochore microtubule attachment that is required to maintain chromosome alignment. It has been reported that CENP-E heterozygosity (haploinsufficiency) increases the level of aneuploidy in primary murine embryonic fibroblasts (MEFs) that are $p19ARF^{-/-}$ (p14ARF in human) (Silk A.D. et al., 2013). Here, we show that 50% reduction of CENP-E induced aneuploidy in nearly diploid HCT116 cells in which p14ARF is not functional. In addition, CENP-E depletion by RNAi triggered aneuploidy associated with reduced levels of p14ARF transcripts in human primary fibroblasts (IMR90). Anyway, after two weeks from CENP-E transcriptional silencing p14ARF expression came back to normal levels in IMR90 cells and aneuploidy decreased. Moreover, the ectopic expression of p14ARF reduced aneuploidy in HCT116-siCENP-E cells. Collectively, our results suggest that the tumor suppressor p14ARF may have an important role in counteracting proliferation of aneuploid cells.

ON13
Synthesis, structural characterization and antitumor activity of di- and tri-organotin(IV) complexes of D-galacturonic acid.

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Six organotin(IV) (R_2Sn^{2+}, R_3Sn^+; R = Me, *n*-Bu and Ph) complexes of D-galacturonic acid (HGalA), of which the three R_3Sn^+ are new derivatives, have been synthesized and their solid-state and solution-phase investigated by IR, ^1H, ^{13}C and ^{119}Sn NMR spectroscopy. Structural characterization of $R_2SnGalA$ complexes was consistent with literature [1]. In the $R_3SnGalA$ complexes, D-galacturonic acid acts as monoanionic and monodentated ligand. In DMSO solution, for these complexes, the tin atom displays a tetracoordination in an almost regular tetrahedrical geometry. The organotin(IV) complexes have been tested by MTT for their cytotoxic activity on HCT-116 tumor cell line (human colorectal carcinoma) [2]. Except for $Me_2SnGalA$ and $Me_3SnGalA$, which were ineffective, all compounds significantly showed a dose-dependent

anti-proliferative effect. However the tri-**organotin(IV) complexes, differently from the relevant di-organotin(IV) derivatives,** did not exerted a time-dependent effect, suggesting a different cytotoxic mechanism. HGalA at 100 μM did not affect the cell viability at any time of treatment. By calculated IC_{50} values, the cytotoxicity of the complexes followed the order $Bu_3SnGalA$ (0.52±0.04 μM) > $Ph_3SnGalA$ (0.91±0.07 μM) > $Ph_2SnGalA$ (2.54±0.20 μM) > $Bu_2SnGalA$ (4.8±0.34 μM). The cell death induced by these organotin(IV)complexes, was considered to be apoptotic by measuring the exposure of phosphadytilserine to the outer membrane.

[1] N. Bertazzi, G. Bruschetta, G. Casella, L. Pellerito, E. Rotondo, M. Scopelliti, Appl. Organomet. Chem. 2003, 17, 932.
[2] M.A. Girasolo, A. Attanzio, P.Sabatino, L. Tesoriere, S. Rubino, G.C. Stocco, Inorg.Chim.Acta 423 2014, 168-176.

Nanotecnologie (NT)

NT1
Effect of composition of Solid Lipid Nanoparticles on their chemical-physical properties and potential for gene therapy

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In the last years, increasing attention has been paid on gene therapy as promising approach to treat different inherited or acquired diseases. To achieve their therapeutic effects, nucleic acids need to cross several biological barriers, reach the cells and their intracellular targets, being protected from degradation by nucleases present in plasma and intracellular compartments. In addition, high molecular weight and negative charges of nucleic acids make them impermeable to the cellular membranes. Therefore, an efficient delivery system is an essential requirement for successful gene therapy. Cationic solid lipid nanoparticles (SLN) have been recently proposed as non viral carriers for gene therapy, due to their widespread technological advantages, including large scale production, use of biocompatible materials and good storage stability [1, 2]. In this study, different SLNs dispersions have been produced by ethanolic precipitation technique using Precirol ATO 5 as lipid matrix and Brij 76 as non-ionic surfactant. Three distinct cationic lipids were chosen (dimethyldioctadecylammonium bromide (DDAB), cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC)), in order to evaluate their effects on the chemical-physical properties (size, zeta potential, crystallinity) and storage stability of the SLNs. The best nanosystems were further complexed with the EGFP plasmid and their different ability to bind the DNA was evaluated by agarose gel electrophoresis. Finally, in order to have preliminary information about biocompatibility of the cationic SLN, haemolytic tests were carried out incubating each formulations with human erythrocytes.

[1] Bondì M.L. et Craparo E.F.. Solid lipid nanoparticles for application in gene therapy: a review of the state of the art. *Expert Opin. Drug Deliv.*, 2010, 7(1): 7–18.
[2] Bondì M.L. et al.. Novel cationic solid-lipid nanoparticles as non-viral vectors for gene delivery. Journal of Drug targeting 2007, 15(4): 295–301.

NT2
PH-sensitive polymeric nanosystems for Doxorubicin delivery

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Polymeric nanosystems have been largely employed in the tumour therapy because of their potential to bypass many of the serious side effects of classical chemotherapeutic treatments. In order to obtain a specific drug release, PHEA (Poly 2 Hydroxyethyl Aspartamide) nanocarriers have been synthesized and conjugated with doxorubicin through a pH-sensitive linker named citraconylamide (PHEA-EDA-P,C-DOXO). It is well known that the tumour microenvironment is more acid (pH 6.4) respect the healthy tissue (pH 7.4) so that this difference can be exploited for a controlled delivery strategy. For this purpose, *in vitro* experiments were carried out by comparing the PHEA-Doxo effect on normal (HB-2) and tumour (MDA-MB-231) cells of the same tissue (human breast tissue). Viability assay showed that PHEA-Dox nanosystems were more cytotoxic to tumour than normal cells. Moreover, uptake studies were conducted by flow cytometry using a fluorescent variant of PHEA-DOXO (PHEA-EDA-P,C-DOXO-FITC). The results revealed that tumour cells presented higher intracellular amount of nanoconjugates, compared with HB-2 cells, suggesting more affinity for cancer condition, carrying to a faster and greater Doxorubicin release in MDA-MB231. These data were also confirmed by co-culture studies, in which it was observed a specific localization of PHEA-DOXO nanosystems in cancer cells after short incubation times. In addition, it was clarified the specific endocytic pathway involved in the PHEA uptake and even the specific localization inside the cell by studying the lysosomal trafficking. These results allowed us to hypothesize a model of pH-sensitive drug delivery system mediated by PHEA nanoconjugates.

NT3
Fluticasone propionate-loaded polymeric nanoparticles as potential drug delivery systems to the lungs

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The local application of corticosteroids by nanomedicine represents an innovative approach for improving the management of lung diseases, as it allows to obtain higher local drug concentrations, constant levels of drug for a prolonged time and reduced side effects due to lower systemic exposure [Smola M et al, Int J Nanomed 3, 1 (2008)]. Here, α,β-poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA) was subsequently functionalised with poly(lactic acid) (PLA) (DD_{PLA}= 4.84 mol%) and with polyethylenglycol (PEG) (DD_{PEG}= 3.5 mol%) to obtain PHEA-PLA-PEG_{2000} graft copolymer [2]. Fluticasone propionate (FP)-loaded polymeric nanoparticles, characterized by nanometer size and slightly negative zeta potential, have been obtained by this copolymer by high pressure homogenization-solvent evaporation method, with a final drug loading of 2.9 wt% [Craparo EF et al, J Nanopart Res 12, 2629 (2010)]. In order to evaluate the biocompatibility of empty and FP-loaded PHEA-PLA-PEG_{2000} nanoparticles, in vitro two different assays were performed on human bronchial epithelial cell line (16-HBE). In particular, we evaluated the cytotoxicity by MTS viability assay, and neither empty nor FP-loaded nanoparticles at different concentrations has no cytotoxicity. Moreover, we evaluated apoptosis and necrosis by annexin-V assay, and neither empty nor FP-loaded nanoparticles at different concentrations induced apoptosis or necrosis. These assays evidence the high biocompatibility of obtained nanoparticles and represents the first step of further study to assess the biological efficiency of this new drug delivery system.

NT4
Synthesis, characterization and antimicrobial activity of polyaminocyclodextrin-capped Ag Nanoparticles

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Biocompatible Ag nanocomposites were prepared by photoreduction of ammoniacal silver acetate in the presence of a polyaminocyclodextrin, namely the poly-{6-[3-(2-(3-aminopropylamino)-ethylamino)-propylamino]}-(6-deoxy)-b-CD (**amCD**, figure 1). The obtained Ag-amCD systems, which possess an oniontype structure [1] with a metal core surrounded by several layers of the capping agent, were characterized by means of various complementary techniques. In particular, FT-IR spectroscopy confirmed the presence of the amCD scaffold in the composite, and evidenced a partial oxidative degradation of the polyamine branches,due to the fact that these groups function as sacrificial reducing agents in the photoinduced process of formation of the Ag metal core. TEM and SAED micrographs evidenced that the Ag cores possess a relatively low polydispersity and a significantly crystalline character.Then, in consideration of the well-known antimicrobial activity of nanosized silver, our **Ag-amCD** systems were assayed for antibacterial activity, quantified as the minimal concentration inhibiting at least the 90% of bacterial growth (MIC90), using Escherichia coli and Kocuria rhizophila as Gram-negative and Gram-positive tester strains. This analysis revealed 5 and 1 μg/ml as MIC90 values against E. coli and K. rhizophila; respectively. In addition, thanks to their peculiar features, the systems function as potential supramolecular drug carriers, effectively able to bind the b-lactam antibiotic Ampicillin (amp, figure 2) as demonstrated by polarimetric measurements. Antimicrobial assays reveals a five-fold improved activity of

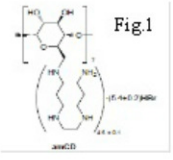
Fig.1

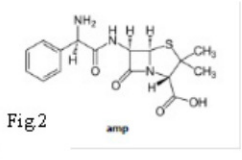
Fig.2

Ag-amCD-amp probably due to synergistic action of Ag nanoparticles and amp. This study provides insights on the attractive possibility to use an environmentally-friendly methodology to produce bioactive supramolecular systems to be employed as powerful and tunable antimicrobial agents.

[1] M. Russo, F. Armetta, S. Riela, D. Chillura Martino, P. Lo Meo, R. Noto J. Mol. Cat. A 408 (2015) 250-261.

NT5

Selective *in vitro* antileukemic activity (HL-60; K-562; MOLT-4; SR) of new Chloro-propyl-pyrazolo[1,2-a]benzo[1,2,5]triazepinones

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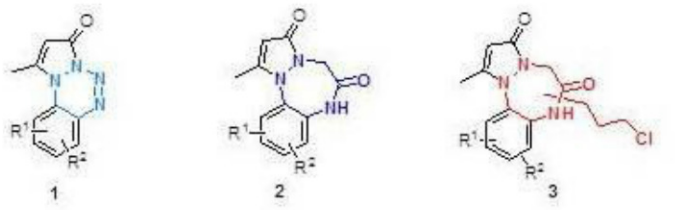

fig.1 Structural modifications carried out on structure 1 leading to selective antileukemic agents

Recently, a new series of pyrazolo[1,2-a]benzo[1,2,3,4]tetrazin-3-one derivatives 1, synthesized and submitted to the NCI one dose screening, showed valuable antiproliferative activity reaching in some cases sub-micromolar values. Further investigations, focused on getting new insights on the mechanism of action, provided further support for their proposition as anticancer candidates. Other than the attractive antiproliferative activity, effective apoptosis induction was observed, together with a cell cycle arrest in S phase (HeLa cell). Thus, encouraged by these results, we planned new structural modifications on structure **1** in order to modulate the biological profile. In detail, a ring expansion was approached. The tetrazine central moiety was replaced with a seven membered ring [1,2,5]-triazepinone one (**2**). Therefore, ten derivatives, variously substituted, were synthesized and submitted to NCI one dose screening. Although literature survey encouraged our research scope because of the wide range of biological properties exhibited by this class of compounds, including anticancer activity, from the NCI screening, only moderate antiproliferative activity was observed. The best value of growth inhibition (GI) was 23% against SNB-75 (CNS cancer), suggesting a central nervous system tropism. Due to the moderate antiproliferative activity showed by the phenyl decorated tricycle (**2**), we decided to introduce a Chloro-propyl side chain on the expanded ring (**3**), as shown in Fig.1 with the aim to increase the activity. The biological outcomes provided by the NCI one dose screening evidenced a selective increased antiproliferative activity only towards leukemic tumor cell lines. Detailed results will be shown on poster session.

NT6

Functionalized nanoparticles with ligands of tumor biomarkers for a diagnostic approach

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Nowadays bioengineering and biomedical research are mainly focused on the development of new technologies to improve diagnosis of complex diseases. Nanotechnology allows the design and manipulation of materials in nanoscale at very low cost, to be utilized in different fields such as clinic diagnosis. Recently, it has been demonstrated that ferritin based nanoparticles (Ft-NPs) are an exceptionally versatile tool both for diagnosis and drug delivery. In this work we will develop Ft-NPs as nanodevice for improving the detection,

sensitivity, speed and accuracy of biomarkers to utilize in clinical tumor diagnosis. By different techniques as immunofluorescence, cytofluorometry, Western blot, we have analyzed the different expression of some biomarkers in different tumor cell lines. In particular we analyzed the level of expression of TrkB Receptor to utilize as neuroblastoma prognostic factor; EP2 and EP4 receptors (for PGE2) to utilize as prognostic factors for lung adenocarcinoma; ICAM-1 and FASL to utilize as lung carcinoma and mesothelioma prognostic factors. On the basis of these results we have planed to functionalize Ft-NPs with the ligands specific for these tumor biomarkers to be employed as diagnostic tools.

NT7
Incorporation of Pt(II) complex with [amino-2-(methylthio)(1,2,4)triazolo-(1,5-a)pyrimidine-6-carboxylic-acid] ligand in MCM41 for controlled release

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Drug carriers play a critical role for the loading and the release of the drug. A promising frontier is represented by a new class of innovative medicines that represents directional transport vehicles "drug delivery" and consist of assembled structures carrier (nano)-drug. Silica-based materials, nontoxic, biocompatible, have been used as adjuvant and excipient in pharmaceutical technology. In this class of compounds, the mesoporous materials, such as MCM41, SBA-15 and hexagonal mesoporous silica, have been investigated for medication and drug delivery due to their properties. In fact, these materials show a large specific pore volume made up of regular pores having a diameter in the nanometer range, which facilitates controlled delivery of pharmaceutical drugs. Recently, a new anticancer drug, the cis-$[PtCl_2(DMSO)HL]\cdot 2DMSO$, where HL = 7-amino-2-(methylthio)[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid, has been synthesized and tested [1]. It exhibited a very marked biological activity on HepG2 hepatocarcinoma cells while under identical conditions it did not affect normal immortalized human liver cells (Chang Liver cells). The scope of this work is to design and investigate a new material constituted by this drug and by mesoporous MCM41 or the MCM41 functionalized with amino group as support. The choice to use the functionalized MCM41 is to investigate the rule of the amino group in the release. The MCM41 was functionalized with amino groups using the grafting method. The incorporation of the drug in the two mesoporous was performed by mixing the two components in chloroform. A detailed characterization of the materials was made using X-ray Diffraction (XRD), FT-IR Spectroscopy, and ^{29}Si Cross Polarization - Magic Angle Spinning NMR (^{29}Si $\{^1H\}$ CP - MAS NMR). The release was evaluated at pH 7.4 and T=37°C in a phosphate buffer solution (PBS) in order to simulate the cellular conditions.

[1] Rubino S., Di Stefano V., Attanzio A., Tesoriere L., Girasolo M.A., Nicolò F., Bruno G., *Inorganica Chimica Acta* 418 (2014) 112-118

NT8

Biocompatible polymers coated Solid Lipid Microparticles (SLM) for pulmonary delivery

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Delivery of high amounts of drugs to the lungs is a desirable goal in the treatment of asthma and chronic obstructive pulmonary disease (COPD). However, increasing the dose of most inhaled drugs may only lead to an increase in side effects, since maximal clinical benefit is usually obtained with the currently recommended dosages. Improving the regional deposition of inhaled drugs may be a more effective way of modifying clinical response [1,2]. Particle size is the most significant determinant of the deposition pattern of inhaled drugs. Optimum drug delivery to the conducting airways occurs with particles ranging from 2.5 to 6 μm. In this work we describe the preparation and the characterization of two different Solid Lipid Microparticles (SLMs) subjected to chitosan and alginato coating for sustained release of fluticasone propionate into the lungs. Due to their mucoadhesive polymeric coating the SLMs could adhere better to the mucous layer on the respiratory epithelium as compared with conventional carriers. The obtained systems are characterized in terms of size, polydispersity index (PDI), zeta potential and morphology. We also evaluated the loading capacity (LC) as well as the kinetics release. Finally, we evaluated the cytotoxicity in vitro of the free FP or entrapped into SLMs and empty microparticles by MTS viability assays on 16-HBE (human bronchial epithelial cells) cell lines. Neither FP-loaded SLMs nor empty SLMs at different concentrations tested showed cytotoxicity compared to the free FP. This assay evidenced the high biocompatibility of obtained microparticles and represents the first step of further studies to asses the biological efficiency of these new drug delivery systems.

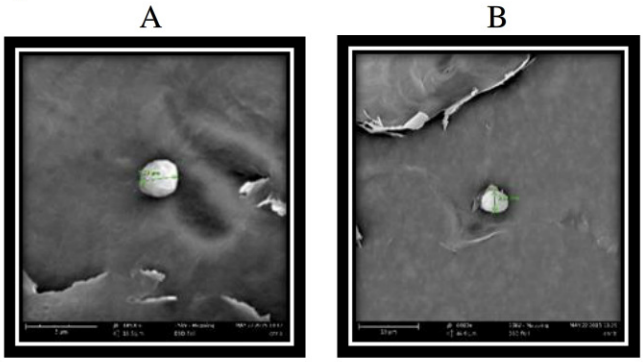

Fig.1 SEM Images of SLM coated with chitosan oligosaccharide (A) and SLM coated with sodium alginate (B).

[1] Bondì M.L. et al.. Effects in cigarette smoke stimulated bronchial epithelial cells of a corticosteroid entrapped into nanostructured lipid carriers. *Journal of Nanobiotechnology*, 2014, 12:46.
[2] Bondì M.L. et al.. Nanostructured lipid carriers as drug delivery systems for fluticasone propionate: Effects in cigarette smoke stimulated bronchial epithelial cells. *European Respiratory Journal*, 2013, 42(57), 223.

Neuroscienze (NS)

NS1

Sustained release of silibinin to the posterior segment of the eye by mucoadhesiveNLC

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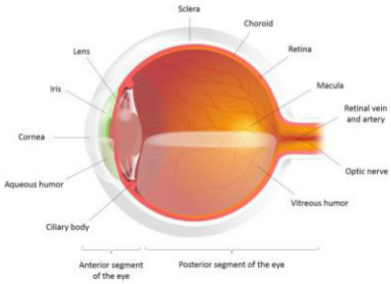

Age-related ocular diseases, such as age-related macular degeneration, diabetic retinopathy and glaucoma, result in life-long functional deficits and enormous global health care costs. As the worldwide population ages, vision loss has become a major concern for both economic and human health reasons. Due to recent research into biomaterials and nanotechnology major advances have been gained in the field of ocular delivery. Drug encapsulation in nanoparticles has been widely investigated and topical applications have recently attracted much attention as it constitutes the only non-invasive route of administration to deliver drugs to the posterior segment of the eye. In this work we describe the preparation and the characterization of a nanostructured lipid carrier (NLC) subjected to chitosan coating for the sustained release of silibinin (SLB) to the back of the eye. Due to its mucoadhesive polymeric coating the NLC could adhere better to the mucous layer of ocular surfaces compared with conventional carriers. The obtained systems are characterized in terms of size, polydispersity index (PDI), zeta potential. We also evaluated the loading capacity (LC) as well as the release kinetics. For cell biocompatibility and biological activity of NLC carrier, we employed the human retinal pigment epithelium cells (ARPE19), which are used for drug permeation studies. Free SLB (0.01-100 μM), NLC or SLB-loaded NLC were added to ARPE cells before or after the insult with H_2O_2, and cell viability was monitored by cell morphology and LDH release assays. No citotoxicity was detected after a 20-hour or 40-hour incubation within 0.01-10 μM concentration range. Cell protection from H_2O_2-induced cell death occurred following pre-treatment and, most importantly, post-treatment with SLB or SLB-loaded NLC. Therefore, SLB-loaded NLC appears to effectively promote cell survival from oxidative stress, and its comparable effects to free-SLB are indicative of NLC ability as a deliverable carrier, albeit more studies are needed.

NS2

TRAIL and TRAIL neutralizing antibody in the retina and RPE/choroid of the 3xTg-AD mouse model of Alzheimer disease

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In Alzheimer's patients, cognitive and memory impairments are accompanied by visual disturbances linked to primary visual cortex dysfunction and retinal degeneration. Features of Alzheimer's disease (AD), such as amyloidogenesis, inflammation and vascular modifications propagate to the retina, as well as, to the brain. Therefore, the retina as a window to the brain may provide important clues to the understanding of AD pathogenesis. There is also a current trend for using the clinical, non-invasive imaging analyses of the retina in order to detect and diagnose AD in patients. Here, we investigated the cytokine TRAIL of the TNF family, which is involved in inflammatory/immune and angiogenic processes, and neurodegenerative events. TRAIL mediates stimuli noxious to neurons, such as amyloid-β (Aβ), ischemia, or trauma, all causing neuronal damage and death. Using a triple transgenic mouse model of AD (3xTg-AD mouse), which bears mutations

in APP, PS1 and tau, and shows pathological signs resembling those of AD patients, we studied the pattern of AD-associated retinal degeneration, extending analysis to the retinal pigment epithelium (RPE) and choroid. They were examined deposition of amyloid-β and phospho-tau, TRAIL and its receptors, activated gliosis and microglia, cytokines and COX-2 expression and, finally, the hypoxia-induced angiogenic VEGF factor and apoptotic death. Importantly, pathological signs of 3xTg-AD retinal specimens were compared to those from 3xTg-AD mice that were subjected to a chronic treatment with an anti-TRAIL antibody for 15 months. The results show Aβ and phospho-tau deposits in the parenchyma and blood vessels of eye structures along with changes in TRAIL system, inflammation and vascular abnormalities, that were all prevented by the treatment with the neutralizing TRAIL antibody. We suggest that the 3xTg-AD mice have AD-associated signs of retinal degeneration and RPE/choroid dysfunction, and TRAIL is central to the aberrant modifications of these structures that benefit from anti-TRAIL treatment.

NS3
Developing rat brain as well as cultured astrocytes contain H1° mRNA-protein complexes

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RNA-binding proteins (RBPs) regulate intracellular transport, pre-localization, stability, and translation of mRNAs [1]. We previously identified a set of proteins which interact with mRNAs encoding H1° and H3.3 histones [2-5]. All these proteins are probably part of a ribonucleoprotein particle [6]. Here we report the results of a more detailed study on the expression and intracellular localization of some of these RBPs, such as hnRNP K and A1, and Hsc70, during rat brain development and in cultured rat astrocytes. We also investigated the presence in the complexes of PIPPin/CSD-C2 protein. Affinity chromatography was performed as already described [6]. Preparation of total lysates and cellular sub-fractions was done as reported in [3]. Possible co-localization of Hsc70 with CSD-C2 in cultured astrocytes was analysed by immunofluorescence microscopy. The presence of Hsc70 chaperone in the already identified ribonucleoprotein complex [6] was confirmed by affinity chromatography. We also found that the complex itself is present not only in the nuclear extracts, but also in the cytoplasmic fraction. Moreover, A1 hnRNP, previously found in the complexes, was found to be differentially expressed and localized during rat brain maturation. We also found that nuclear levels of A1 increase in cultured astrocytes grown on a fibronectin-containing substrate, but decrease when cells are fed with Maat medium [7]. We finally report that sumoylated PIPPin, already found in neurons, is also present in the nuclei of cultured astrocytes.

[1] Di Liegro et al. 2014, Int J Mol Med 33:747-62.
[2] Scaturro et al. 1998, J Biol Chem 273:22788-91.
[3] Nastasi et al. 1999, J Biol Chem 274:24087-93.
[4] Sala et al. 2007, Int J Mol Med 19:501-9.
[5] Saladino et al. 2012, Int J Mol Med 29:141-5
[6] Di Liegro et al. 2013, Neuroscience 229:71-6.
[7] Cestelli et al., 1985, Brain Res 354:219-27.

NS4
Effect of a natural liver protector supplement in the brain

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Several evidences indicate obesity as a risk factor for neurodegenerative diseases. In obesity status insulin signaling is inhibited both in the liver and brain by impairing the signaling cascade at multiple levels and inducing insulin resistance, oxidative stress and inflammation. It has been postulated that cytotoxic ceramides transferred from the liver to the blood can enter the brain due to their lipid-soluble nature, and thereby exert neurodegenerative effects via a liver–brain axis. Thus, using a liver protector supplement, a benefit both for liver and brain should be expected. KEPAR is a commercial natural supplement containing several plant extracts such as turmeric (*Curcuma longa*), silymarin (*Sylibum marianum*), guggul (*Commiphora mukul*), chlorogenic acid (*Cynara scolymus*), and inulin (*Taraxacum officinale*) and used for liver protection. C57BL6 mice were fed with a standard diet (STD) or with a high fat diet (HFD), to induce obesity, in absence/presence of KEPAR solution, for 4 mouths. After this treatments the mice were weighted and cholesterol, triglycerides and glucose parameters were measured at baseline to confirm occurrence or inhibition of the metabolic syndrome. After these tests mice were sacrificed and the effect on the brain was analyzed. Brain of mice HFD fed showed an increase of ROS and NO generation that was reduced in mice treated with KEPAR. Western blot analysis suggest that the supplement decrease the levels of HSP60, H-Oxy and i-Nos stress inducted proteins. By immunofluorescence studies we have also demonstrated that the supplement have a anti-inflammatory effect in the brain by decreasing the levels of expression of some inflammatory proteins such as a glial fibrillary acidic protein (GFAP). The neuroprotective role of KEPAR was confirmed by *in vitro* studies on LAN5 neuroblastoma cells, in which oxidative stress and inflammation was induced and specific markers analyzed.

NS5
Low concentrations of silibinin and choline-calixarene or INU-C8-PEG carriers assembling silibinin promote antioxidant and antiapoptotic effects in neuronal and retinal epithelial cells

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Silibinin (SLB), the main component *of silymarin* (*Silybum Marianum),* is a flavonoid known for its multiple biological activities. In cancer model, SLB shows antioxidant, anti-inflammatory, anti-cancer and anti-angiogenic activities, and appears to yield neuroprotection in neurodegenerative models. In this study, our first objective was to investigate the effects of SLB in cellular models for oxidative stress mimicking a common pathological mechanism to neurodegenerative diseases, including retinal diseases such as age-related macular degeneration (AMD) and diabetic retinopathy. Three different cell culture types were used: the retinoic acid (RA)-differentiated SY5Y cells as a neuron-like cell model, the human corneal epithelial cells (HCEC) as an in vitro ocular disease model and the human retinal pigment epithelial cells (ARPE19) as a typical model for AMD studies. Undifferentiated SY5Y human neuroblastoma was used as a positive control of anti-cancer SLB effects. As a second objective, due to the hydrophobic nature of SLB decreasing

its bioavailability, two nanocarriers, the choline-calix[4]arene derivative (CALIX) and the polymeric micelles, INU-C8-PEG, both loaded with SLB, were designed to improve its delivery to the retina. Their biological activity was tested on ARPE19 cells, being these cells with its tight junctions a blood-retinal-barrier model for drug permeation studies. Our results show toxic effects of high SLB concentrations (10-100μM) on neuroblastoma cells, but lack of toxicity and prevention of oxidative stress-induced cell death in RA-differentiated SY5Y, HCEC and ARPE19 cells using low concentrations of SLB (0.01-1μM). Analyses of SLB-loaded CALIX or INU-C8-PEG in ARPE19 cells insulted with H_2O_2 or $FeSO_4$ and assessed for viability and apoptotic protein expression reveal a good correspondence between free and SLB-loaded carriers in promoting cell survival. In conclusion, we demonstrate that SLB anti-cancer and protective effects depend on its concentrations, and CALIX and INU-C8-PEG_{2000} are promising carriers for SLB delivery and potential therapy for retinopathies.

NS6
A bicistronic vector to study the effect of CLN8/VAPA interaction on cell physiology and eventually the pathology of CLN8-associated NLC diseases

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The neuronal ceroid lipofuscinoses (NCLs) are rare forms of lysosomal storage diseases but the most common neurodegenerative disorders affecting children. All NCLs are characterized by dementia, epilepsy, and motor decline; in most NCL forms, children also manifest a progressive visual failure. Moreover, they share a common feature to accumulate lipopigments in lysosomes of neural cells and other cell types. However, they differ for the link to distinct genes and various mutations of the same gene that lead to a wide phenotypic variability of disease courses. The exact function of the most encoded proteins is still unclear and needs to be elucidated. Mutations of CLN8 gene lead to two distinct phenotypes: the progressive epilepsy and mental retardation (EPMR), and a variant of late-infantile NCL (vLINCL). CLN8 gene encodes an ER-resident transmembrane protein of 286 a.a.; despite its specific role is unknown, it has been tightly related to lipid homeostasis, specially to sphingolipids. In our laboratory, through a yeast-two-hybrid approach and a human cDNA library screening, it has been drawn a partial interactome. Of the identified CLN8 partners, the VAPA (vesicle-associated membrane protein/VAMP-associated-protein-A) protein is the docking site of ceramide transporter proteins in the ER, working in lipid physiology. EPMR patients and the murine model of vLINCL have impaired levels of ceramide metabolites. Thus, the interaction between VAPA and CLN8 could have a key role in regulating the traffic and physiology of ceramide. To gain insights on CLN8/VAPA interaction, we have prepared a “bicistronic” mammalian expression vector on which both CLN8 and VAPA were expressed under a CMV promoter and in fusion with two different Tags at N terminus. The “bicistronic” construct by overexpressing both proteins in the same cellular contest could help to investigate the functional role of CLN8/VAPA interaction in lipid homeostasis and, so its implication in CLN8 diseases-associated mechanisms.

NS7
CLN8 protein: a post-translation modification and subcellular distribution study for understanding CLN8 physiopathology

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The neuronal ceroid lipofuscinoses (NCLs) are devastating autosomal recessive neurodegenerative diseases of children, belonging to the family of lysosomal storage disorders (LSDs). NCLs show broad clinical and allelic heterogeneity, more than fourteen genes (CLN genes) and 400 mutations are known, and they are characterized by a common feature of intralysosomal accumulation of lipofuscin. Some CLN proteins have

already a function (i.e., cathepsin D, PPT1, and TPP1), others proteins (CLN3, CLN5, CLN6, CLN7, and CLN8) still wait to be defined. Mutations of CLN8 cause two major clinical phenotypes: the progressive epilepsy with mental retardation (EPMR or Northern Epilepsy), a juvenile-onset phenotypic variant, and a more severe form with a late-infantile onset (LINCL). A CLN8 mutation spontaneously occurring in mice results in the motor neuron degeneration (mnd) phenotype. The CLN8 is a ubiquitous membrane protein of 286 a.a., containing an ER-retrieval signal (KKRP) and primary located at the ER. Probable functions of CLN8 implies an involvement in the lipid synthesis and/or proteolipid trafficking or as a lipid sensing. In this study, to elucidate CLN8 protein function we better characterized the subcellular localization and verified its post-translational modification. Endogenous and recombinant protein expression were examined in differentiated and undifferentiated human neuroblastoma cells and in a human epithelial cell line, that were subjected to treatments with specific drugs activating phosphorylation and/or inhibiting phosphatase. Protein localization was also assessed in fractionated lysates of cerebella from wild type and mnd mice. Data obtained indicate a possible CLN8 threonine phosphorylation site which lacks in the sequence of CLN8 mutated in the EPMR disease, thus suggesting its important functional role. Also, hints regarding CLN8 role could be uncovered by a different subcellular localization of various forms of CLN8 under physiological and pathological conditions.

Microrganismi nelle Biotecnologie (MB)

MB1

Role of the two component system Dbv6/Dbv22 in regulating A40926 biosynthesis in *Nonomuraea sp.* ATCC 39727

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The actinomycete *Nonomuraea* sp. ATCC 39727 produces the glycopeptide A40926, precursor of dalbavancin, recently approved by FDA for treatment of severe Gram positive bacterial skin infections. Biosynthesis of A40926 is encoded by the *dbv* gene cluster, which contains 37 protein coding sequences that participate in antibiotic biosynthesis, regulation, immunity, and export. Specifically, the *dbv* cluster contains three regulatory genes, *dbv3*, *dbv4* and *dbv6*. The positive regulatory role of Dbv3 and Dbv4 on A40926 biosynthesis was already demonstrated, while the role of Dbv6 has not yet been elucidated. Dbv6 is a putative response regulator being part of the two component system Dbv6-Dbv22 in which Dbv22 should act as the sensor kinase. Two independent mutants in *dbv6* and *dbv22* were generated. Analysis of the Δ*dbv6* and Δ*dbv22* mutant strains demonstrated that Dbv6 and Dbv22 do not affect the bacterial growth, while both of them negatively control the antibiotic production. In fact, bioassays and LC-MS analyses showed that the Δ*dbv6* and Δ*dbv22* mutant strains produce 2- and 3-fold more antibiotic, respectively, than the parental strain. Quantitative RT-PCR analysis confirmed that some biosynthetic *dbv* genes are more transcribed in both mutant strains and mobility shift assays, conducted with a His-tagged Dbv6 showed that these genes are under the direct control of Dbv6. These results strongly suggest the Dbv6 and Dbv22 work together. Combining these data with previous knowledge, we propose a complex model of antibiotic regulation with three different regulatory mechanisms governing A40926 production.

MB2

Bioactive molecules from soil and marine bacteria: new potential applications

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Microorganisms produce a great amount of enzymes that degrade a wide variety of organic materials such as oils, proteins, and esters. These enzymes are of great importance in the development of industrial

bioprocesses and could be used in several biotechnological fields: bioremediation, feed industry, food processing, cosmetic products and in this study have been tested for enzymatic bio-cleaning of works of art surfaces. Recently, bioactive molecules secreted by soil and marine bacteria showed encouraging results in degrading different substrates. We are analysing different hydrolases secreted by Streptomyces coelicolor, a model actinomycete, and by marine bacteria from Mediterranean Sea. The enzymatic activity of these hydrolases was tested through zymography using as substrate: gelatin and casein for proteases, 4-Nitrophenyl myristate for esterases and olive oil for lipases, respectively. Noteworthy, these novel enzymes show a good hydrolase activity at different temperatures, including temperatures below 30°C, unlike the commercial enzymes that usually need higher temperatures (≥ 37 °C). Since these enzymes operate with high selectivity and at temperature < 30°C, they could be utilized in different bioprocess such as the enzymatic bio-cleaning of art surfaces, where a crucial point is represented by the temperature of application.

MB3
Isolation and partial characterization of methanogenic bacteria from a digester fed with vegetable biomass and swine manure

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The use of renewable energy sources is becoming increasingly essential, in order to reduce emissions from fossil fuel sources that have impact on global warming. Therefore, biomass presents one of the most common form of renewable energy source for feasible utilization; it is widely available, and the energy produced can widely reduce carbon dioxide emissions. Biomass can biologically be converted to biogas (CH4, CO2). The anaerobic digestion is one of the most economic way to produce biogas from various biomass substrates; during this process different consortia of microorganisms are needed. The methanogenic archea play main role in the process of methane gas production. The aim of our project is the isolation and characterization of methanogens species present in samples from a biodigester containing vegetable biomass and swine manure. Some enrichment cultures of methanogens, through the use of anaerobic techniques, were obtained in our laboratories. Analysis of methane production, carried out using the ABB A2020 Advance Optima process gas analyzer, showed that the formate served as a substrate for the methanogenesis. We also performed PCR analysis of 16S rRNA partial sequence and mcrA gene to confirm the presence of various methanogenic bacteria. The next step is to characterize the individual micro-organisms present in the enrichment cultures. The final purpose is to better understand the metabolism of these bacteria and also to improve the efficiency of biogas production.

MB4
The seed microbiota of *Anadenanthera colubrina*

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Although the importance of plant-associated microorganisms for plant growth and health was getting more recognition recently, the role of seed-associated microorganisms, and especially seed endophytic bacteria, still is underestimated. Nevertheless, these associations could be beneficial for germination and seedling establishment as seed endophytic bacteria are already present in these very early plant growth stages. In this

work, the presence of endophytic bacteria in the seeds of *Anadenanthera colubrina* var. *cebil* (Vell.) Brenan, a Fabaceae tree of South America, has been demonstrated using culture-dependent, culture-independent approaches and FISH analysis. Culture-dependent approach, based on the isolation of cultivable bacteria and subsequent identification by analysis of their 16S rDNA sequences, allowed the identification of species closely related to *Staphylococcus* and *Methylobacter.* The culture-independent approach, based on the high-throughput sequencing 16S rDNA amplified from seed metagenomic DNA, confirmed the results of the culture dependent approach. This analysis also identified *Actinomyces*, *Pseudomonas* and *Clostridium* genera. Fluorescence in situ Hybridization coupled with Confocal Laser Scanning Microscopy (FISH-CLSM) confirmed the presence of bacteria belonging to both Alphaproteobacteria and Firmicutes Phyla in seed cryosections. The bacteria were arranged as single cells or small colonies with up to ten cells and colonized the outer seed coat as well as the internal tissues. All these results demonstrate for the first time that typical Phyla of the root- and leaf-microbiota, such as α Proteobacteria, Firmicutes and Actinobacteria, are present in the seeds of *Anadenanthera colubrina.*

MB5
Dicationic imidazolium salts: tunable antimicrobial and antitumoral chemotherapeutic leads

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The chemical synthesis of novel chemotherapeutical leads is evolving thanks to possibility to design molecules with desired physical-chemical and, thus, biological properties. The imidazolium salts, recently proven effective to inhibit bacterial and/or cancer cell growth, possess an amphiphilic nature that is conferred by the imidazolium cation having a polar head generally coupled with aliphatic side chains. Thus, biological properties of imidazolium salts can be tuned through modifications involving the cation structure and/or the anion nature. By covalently linking two imidazolium rings, diimidazolium salts were obtained differing in: i) kind of anions; ii) isomeric cations or anions; iii) length of the imidazolium alkyl side chains. Initially, eleven diimidazolium salts, differing for their anionic counterparts, were assayed for: i) antibacterial property, quantified as the minimal concentration inhibiting at least the 90% of bacterial growth (MIC90) using Escherichia coli and Kocuria rhizophila as Gram-negative and Gram-positive tester strains respectively; ii) antitumoral activity measured as the concentration inhibiting the 50% of cell growth (IC50) using SKBR-3 breast cancer cell line. All the assayed diimidazolium salts possess biological activity showing i) 0.1-0.5 and 25-50 μg/ml as MIC90 values against of K. rhizophila, and E. coli; respectively, and ii) 30-55 μg/ml as IC50 values. Among the tested diimidazolium salts, three were chosen to further investigate the relationship between biological efficacy and either length of alkyl side chains or isomeric substitution on the cation. Isomeric substitution revealed few or no effect while a positive correlation between alkyl chain length and cell-growth inhibitory efficacy was shown. Although further studies have to be performed to elucidate the molecular mechanisms leading to cell growth arrest, this study provides insights on the attractive possibility of dicationic imidazolium salt exploitation as chemotherapeutic compounds whose activity can be tuned by modifying structural characteristics.

MB6
Medieval grape seeds: morphometrical and molecular investigations

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Grape cultivation (*Vitis vinifera* L. ssp. v*inifera*) in the southern Italy is dated to Middle Bronze/Iron Age, while in Sicily it was presumably introduced by Greek settlers. Nevertheless, grape diffusion is motive of debate. Grape seeds are usually found during the archaeological excavations and their study is very useful for reconstructing the diffusion of this species. The *status* of seed preservation depends from the taphonomy of the site, and they can be waterlogged, dried, mineralizated and more or less crushed. Morphological and molecular approaches must work together to clarify the history of *Vitis.* In this work we present preliminary results on both morphometrics and genetics analysis of ancient seeds collected from a Middle-Aged underground well in Palermo urban area. Sicilian ancient seeds showed a characteristic shape that differs from others already described in archaeological sites. The analyzed indices (Stummer and Perret) collocated the ancient seeds more closed to the wild grape [*V. vinifera* L. subsp. *sylvestris* (Gmelin) Hegi] distribution. The ancient DNA (aDNA) was successfully extracted using both phenol-chloroform method or CTAB protocol although with low yield (8-20 ng/µl). PCR amplification products of rbcL gene (60-100 bp) were obtained using *ad hoc* designed primers, the nucleotide compositions were determined and the sequences analysis are in progress.

MB7
Antibacterial activity of biocidal compounds loaded on mesoporous materials

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Biodeterioration represents one of the main causes of deterioration of archaeological materials, monuments and works of art, especially in humid environments. Besides insects, mosses and lichen, microorganisms contribute significantly to the overall deterioration phenomena. Biological decay is often encountered with biocides. These prove to be efficient in reducing the microbial fauna. However, the biocides usually act only in limited periods of time, as they can as well deteriorate or be washed out by humidity. Therefore treatment with biocides often requires frequent application. This comes hand in hand with high costs and hazards to the conservator, the environment and eventually even the object. MCM41 mesoporous silica, hexagonally ordered, shows a large specific pore volume made up of regular pores having a diameter in the nanometer range, which facilitates the controlled delivery of drugs and several kinds of molecules. Its surface can be modified with functional groups in order to modify the physical and chemical interactions between the support and the embedded molecules, thus allowing a controlled release of this in a medium. The aim of this study is to design a system constituted by MCM41/biocide which slowly release the biocide in a constant and controlled manner. This system could be used by restorers to reduce the frequency and quantity of biocide application in order to reduce the aforementioned risks. MCM41 and MCM41 functionalised with carboxy- (MCM41-COOH) and amino-groups (MCM41-NH2) are used as support. Biotin T and NewDes 50, commercially available, are used as biocides. The effect of both surface modifications on the release of biocides was tested, as well as the biological activity of the system. The application of good performing systems was tested in two case–studies (paper and stone).

MB8

A small protein is involved in tryptophan biosynthesis and morpho-physiological differentiation in Streptomyces coelicolor

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Streptomycetes, bacteria belonging to the phylum of Actinobacteria, are characterized by a complex life cycle (which includes the formation of a vegetative mycelium, an aerial mycelium and spores) and the production of many secondary metabolites, including antibiotics. At the cellular level, regulatory factors comprise regulatory proteins, small RNAs and small ORFs. It is known that small ORFs (smorfs) can regulate the translation of downstream elements and can even encode functional peptides involved in the regulation of specific pathways. In the model streptomycete S. coelicolor, smorfs (100-300 nucleotides) were identified in biosynthetic amino acid gene clusters, such as trpM in tryptophan's one. Previous phenotypic and proteomic analysis of a trpM-knockout mutant strain, revealed that TrpM, a small protein of 63 amino acids, is involved in tryptophan metabolism and in morpho-physiological differentiation. In this work we describe the construction and the characterization of a trpM knock-in mutant strain. This strain shows an earlier production of the aerial mycelium and an increased production of CDA (calciumdependent antibiotic) and actinorhodin antibiotics in comparison to the wild type strain. Moreover, it produces a larger amount of spores, as revealed by SEM analysis and spore quantification. All these results confirm the key role of TrpM in S. coelicolor tryptophan metabolism and morphophysiological differentiation. Moreover, the over-expression of trpM could be regarded as a novel strategy to increase antibiotic production in Streptomyces strains of industrial interest

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