UNIVERSITÀ DEGLI STUDI DI PALERMO

DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Istituto di Biomedicina e Immunologia Molecolare "Alberto Monroy" - CNR

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Ricerca Biologica e Biotecnologica

Congresso Scientifico:

Ricerca di base, interdisciplinare e traslazionale in ambito Biologico e Biotecnologico (II ed.)

26 e 27 Giugno 2014

Aula Mutolo della Sezione di Biologia Cellulare del Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF)

In copertina presentiamo una nuvola di tag (tag cloud in Inglese), rappresentazione visiva delle etichette (tag) o parole chiave usate negli abstract dei lavori del Congresso.
Generalmente questa lista è presentata in ordine alfabetico, con la peculiare caratteristica di attribuire un font più grande alle parole più importanti. Si tratta quindi di una lista pesata.
Le nuvole di tag costituiscono un elemento di interfaccia per gli architetti dell'informazione, che le possono utilizzare per progettare navigazioni alternative all'interno di un sito web.
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ISBN 9788890580550

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Aula Mutolo della Sezione di
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Biologiche, Chimiche e Farmaceutiche (STEBICEF)

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Anche quest'anno, il Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF) dell'Università di Palermo e l'Istituto di Biomedicina e Immunologia Molecolare (IBIM) del CNR di Palermo promuovono un convegno scientifico congiunto.

Il convegno, dal titolo "Ricerca di Base, Interdisciplinare e Traslazionale in ambito Biologico e Biotecnologico", avrà luogo il 26 e 27 Giugno 2014 presso l'Aula Mutolo della Sezione di Biologia Cellulare del Dipartimento STEBICEF, in viale delle Scienze, Edificio 16.

Tale evento si innesta pienamente nel contesto della convenzione Università-CNR, proponendo uno scambio interculturali mirato a diffondere lo stato dell'arte delle ricerche condotte dai componenti dei due Enti.

Il convegno offre inoltre un'importante occasione di confronto e di incontro anche per colleghi che operano in altre Strutture.

Durante lo svolgimento dei lavori i partecipanti avranno anche l'occasione di trovare momenti di approfondimento sulle tematiche proposte (quali Biologia Molecolare, Biochimica, Biologia dello Sviluppo, Genetica, Fisiologia, Microbiologia e molte altre ancora), sia da un punto di vista prettamente metodologico che per quanto attiene la nascita di nuove e proficue collaborazioni.

Per raggiungere tali obiettivi, il convegno si articola alternando due tipologie di sessioni: una inerente le comunicazioni orali e l'altra l'esposizione di poster.

Al fine di promuovere la divulgazione delle attività, tutte le comunicazioni scientifiche sono incluse negli Atti.

Il Comitato Scientifico

Dr. Vincenzo Cavalieri
Dr. Davide Corona
Dr. Marta Di Carlo
Dr. Mirella Ciaccio

Congresso "**Ricerca di base, interdisciplinare e traslazionale in ambito Biologico e Biotecnologico**"

Presso l'Aula Mutolo del Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche

26-27 Giugno 2014

PROGRAMMA

Giovedì 26 Giugno

8.30 REGISTRAZIONE
9.15 SALUTO DI BENVENUTO E APERTURA DEI LAVORI
Prof. ***Giovanni Spinelli***, Direttore STEBICEF - UNIPA
Dr. ***Giovanni Viegi***, Direttore IBIM – CNR

9.40 - 11.00 Sessione I
Moderatori: Dr. Vincenzo Cavalieri / Dr. Maria Di Bernardo

9.40 – 10.00 **Santa Anna Acuto**, A.O. Ospedali Riuniti Villa Sofia-Cervello
The sea urchin *sns5* chromatin insulator settles a gene therapy vector into an independent domain of expression in the vertebrate genome.

10.00 – 10.20 **Salvatore Molino**, STEBICEF - UNIPA
Thanatos associated protein 11 (THAP11) modulates expression of c-MYC by binding the HB2.8 enhancer blocker element

10.20 – 10.40 **Maria Cristina Onorati**, STEBICEF - UNIPA
Chromatin remodelers, nucleoplasm compartment and proteinopathies

10.40 – 11.00 **Giosalba Burgio**, STEBICEF - UNIPA
UbcD1 is a Histone H2B Ubiquitin-Conjugating Enzyme Essential for Global Chromatin Structure and Gene Expression Regulation

11.00 - 11.30 ***Coffee break / visione Poster***

11.30 - 12.50 Sessione II
Moderatori: Prof.ssa Anna Maria Puglia / Dr. Mirella Profita

11.30 – 11.50 **Giulia Anzalone**, IBIM – CNR
IL-8 and TSLP production from epithelial cells in IL-17A mediated airway inflammation of COPD patients.

11.50 – 12.10 **Giovanna Barbieri**, IBIM - CNR
The growth inhibition of $(Bu_3Sn)_4TPPS$ and $(Bu_2Sn)_2TPPS$ treated human melanoma cells is associated to decrease of adhesion receptors expression

12.10 – 12.30 **Teresa Faddetta**, STEBICEF - UNIPA
Metabolic Pathways in *Microbispora sp.* ATCC-PTA 5024, Producer of NAI-107 Lantibiotic

12.30 – 12.50 **Giovanna Barresi**, STEBICEF - UNIPA
Biotecnology and Cultural Heritage: bioactive molecules applied in restoration projects

12.50 - 14.30 ***Light Lunch / visione Poster***

14.30 - 16.10 Sessione III
Moderatori: Dr. Melchiorre Cervello / Prof. Aldo Di Leonardo

14.30 – 14.50 **Daniela Carlisi**, BIONEC – UNIPA
The synergistic effect exerted by the HDAC inhibitor SAHA and the sesquiterpene lactone parthenolide on triple negative breast cancer cells.

14.50 – 15.10 **Gaetano Felice Caldara**, STEBICEF - UNIPA
How cancer cells cross lymphatic endothelium?

15.10 – 15.30 **Maria Rita Emma**, IBIM - CNR
Role of Nupr1/p8 in hepatocellular carcinoma: implications in cell growth control and response to treatment

15.30 – 15.50 **Walter Arancio**, DIBIMIS - UNIPA
Anaplastic Thyroid Carcinoma: a ceRNA analysis pointed to a crosstalk between *SOX2*, *TP53* and microRNA biogenesis.

15.50 – 16.10 **Riccardo Di Fiore**, STEBICEF - UNIPA
microRNA-29b-1 is involved in self-renewal and fate decisions of human osteosarcoma 3AB-OS cancer stem cells

16.10 - 18.00 ***Coffee break/ visione Poster***

Venerdì 27 Giugno

09.00 - 10.40 Sessione IV
Moderatori: Dr. Maria Grazia Zizzo / Dr. Giovanni Duro

09.00 – 09.20 **Michelangelo Auteri**, STEBICEF - UNIPA
Novel evidences for a role of dopamine as modulator of intestinal motility: a study on mouse distal colon.

09.20 – 09.40 **Domenico Nuzzo**, IBIM - CNR
Diet-Induced Obesity: A Risk Factor for Alzheimer's disease

09.40 – 10.00 **Carmela Zizzo**, IBIM – CNR
Malattia di Anderson Fabry: misdiagnosi e nuovi marcatori molecolari

10.00 – 10.20 **Rita Messineo**, IBIM - CNR
Relationship between Human alfa-Galactosidase Isozymes

10.20 – 10.40 **Antonella Amato**, STEBICEF - UNIPA
Chronic treatment with GLP-2 (3-33) exacerbates glucose metabolism disorders in mice fed a high fat diet.

10.40 – 11.10 ***Coffee break / visione Poster***

11.10 - 12.50 Sessione V
Moderatori: Prof. Giulio Ghersi / Dr. Antonella Bongiovanni

11.10 – 11.30 **Rosa Alduina**, STEBICEF - UNIPA
Streptomyces coelicolor: DNA methylation and differentiation

11.30 – 11.50 **D Spigolon**, IBF - CNR
Hsp60 and GroEL Chaperonins: Thermodynamic Characterization on Self-Assembly and Structural Stability Studied by Nano DSC and Nano ITC

11.50 – 12.10 **Patrizia Cancemi**, STEBICEF - UNIPA
A proteomic signature for breast cancer patients stratification

12.10 – 12.30 **Loredana Randazzo**, IBF - CNR
Protein diffusion in ovo

12.30 – 12.50 **Patrizia Saladino**, STEBICEF - UNIPA
RNA binding proteins in brain cells differentiation

12.50 – 14.00 ***Light Lunch / visione Poster***

14.00 - 15.20 Sessione VI
Moderatori: Dr. Giovanna Barbieri / Dr. Fabiana Geraci

14.00 – 14.20 **Pasquale Picone**, IBIM - CNR
NANOGELS AS USEFUL TOOL FOR ALZHEIMER'S DISEASE THERAPY

14.20 – 14.40 **Angelo Spinello**, STEBICEF - UNIPA
The Interaction of Small Molecules with Biomolecules

14.40 – 15.00 **Nicolò Mauro**, STEBICEF - UNIPA
Clever pH-Sensitive Drug-polymer Conjugates For Targeted Cancer Therapy

15.00 – 15.20 **Vincenzo Martorana**, IBF - CNR
A molecular strategy to cope with serpinophaties.

15.20 Premiazione migliori Poster.

Ore 15.30 Chiusura dei lavori

Abstract comunicazioni orali:

Chronic treatment with GLP-2 (3-33) exacerbates glucose metabolism disorders in mice fed a high fat diet.

Antonella Amato[1], Sara Baldassano[1], Flavia Mulè[1].
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presenting author: antonella.amato@unipa.it
Keywords: **Obesity, glucagon like peptide-2, glucose metabolism**

BACKGROUND AND AIM: Glucagon like peptide-2 (GLP-2) is a gastrointestinal hormone released in response to dietary nutrients, particularly carbohydrates and fats and acting through a specific G protein-coupled receptor, the GLP-2 receptor (GLP-R). The physiological effects of GLP-2 are multiple, including its intestinotrophic action. We have recently showed that GLP-2 is involved in the intestinal adaptation to high fat diet (HFD). Indeed, in HFD fed mice, the block of the GLP-2 signaling reduces the increase in crypt–villus height and in the cell number per villus that occurs following a chronic HFD. In consideration of the well known relationship between chronic HFD and impaired glucose metabolism, in the present study we examined if the blocking of the GLP-2 signaling leads to functional consequences in the regulation of glucose metabolism in HFD-fed mice.
METHODS: Fasting glucose and insulin, intraperitoneal glucose tolerance, insulin sensitivity, and plasma insulin levels after intraperitoneally (i.p.) glucose load, were examined in C57BL6/J mice fed a standard diet (STD) or HFD at the tenth week. The same parameters were evaluated in mice fed HFD or STD treated once a day with i.p. GLP-2 (3-33), a GLP-2 receptor antagonist (60 ng), or PBS (vehicle control) for four weeks (from the sixth to the tenth week).
RESULTS: Compared with age-matched control animals, HFD fed mice exhibited hyperglycemia in fasted and glucose-stimulated states. None difference was seen in fasting plasma insulin levels while insulin was significantly more elevated during glucose tolerance test. No difference in glucose excursion was detected after i.p. insulin injection.
In HFD fed mice, GLP-2 (3-33) did not modify fasting hyperglycemia, but significantly decreased the i.p. glucose tolerance, increased fasting and glucose-induced insulin levels, and reduced the sensitivity to insulin leading to insulin-resistance.
In STD fed mice, chronic treatment with GLP-2 (3-33) did not affect any parameters.
CONCLUSIONS: This study suggests that endogenous GLP-2 may play a role in the adaptation to a HFD because the block of the GLP-2 signaling worsens the glucose homeostasis.

IL-8 and TSLP production from epithelial cells in IL-17A mediated airway inflammation of COPD patients.

Giulia Anzalone[1], Loredana Riccobono[1], Anna Bonanno[1], Giusy Daniela Albano[1], Angela Marina Montalbano[1], Caterina Di Sano, Rosalia Galiardo[1], Mark Gjomarkaj[1], Liboria Siena**,** Mirella Profita.[1]
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Keywords : **airway inflammation, lung epithelial cells, interleukin 17A**.

IL-17A plays a key role in the persistence of airway inflammation, oxidative stress, and in the reduction of steroid-sensitivity in COPD. Very few studies describe IL-17A activities in airway inflammation during COPD. We measured the levels of IL-17A in sputum supernatants (ISSs) from healthy controls (HC) (n=10), healthy smokers (HS) (n=10), and COPD patients (n=10). Furthermore, human bronchial epithelial cells (16HBE) were stimulated (4 hrs and 24 hrs, 37°C) with ISSs from HC (n=6), HS (n=6), or COPD (n=6), as well as with human recombinant (hr) IL-17A. IL-8 and TSLP were evaluated in 16HBE supernatants and in cell lysates by ELISA and by WB, respectively. HDAC2 activity was evaluated in nuclear cell lysates by a commercial colorimetric assay kit.

IL-17A was increased in ISSs from COPD patients and HS subjects when compared with HC. IL-8 and TSLP were higher in cell lysates and supernatants of 16HBE stimulated with ISSs from COPD and HS than in cell lysates and supernatants of 16HBE stimulated with ISSs from HC and in the cells stimulated with hrIL-17A when compared with untreated cells. HDAC2 activity was reduced in nuclear cell lysates of 16HBE stimulated with hrIL-17A when compared with untreated cells.
Our findings suggest that IL-17A present in the airway of COPD patients is able to increase the IL-8 and TSLP production due to a markedly reduction of the HDAC2 activity.

Anaplastic Thyroid Carcinoma: a ceRNA analysis pointed to a crosstalk between *SOX2*, *TP53* and microRNA biogenesis.

[1]Walter Arancio, [1,2]Valeria Carina, [1]Giuseppe Pizzolanti, [1]Laura Tomasello, [1]Maria Pitrone, [1]Concetta Baiamonte, [1]Marco Amato, [1]Carla Giordano*.
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Keywords: **Anaplastic thyroid carcinoma, Stem cells, RNA interference**.

Cancer stem cells (CSC) may play a central role in oncogenesis, especially in undifferentiated tumours (1).
Anaplastic Thyroid Carcinoma (ATC) has characteristics suggestive of a tumour enriched in CSC (2).
Previous studies suggested that the stem cell factor *SOX2* has a pre-eminent hierarchical role in determining the characteristics of stem cells in SW1736 ATC cell line. In detail, silencing SOX2 in SW1736 is able to suppress the expression of the stem markers analysed, strongly sensitizing the line to treatment with chemotherapeutic agents (3).
Therefore, in order to further investigate the role of SOX2 in ATC, a competing endogenous RNA (ceRNA) analysis (4) was conducted in order to isolate new functional partners of SOX2. Among the interactors, of particular interest are genes involved in the biogenesis of microRNAs (*DICER1*, *RNASEN*, *EIF2C2*), in the control cell cycle (*TP53*, *CCND1*), and in mitochondrial activity (*COX8A*).
The data suggest that stemness, microRNA biogenesis and functions, p53 regulatory network, cyclin D1 and cell cycle control, together with mitochondrial activity, might be co-regulated.

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2. Hardin, H, et Al. (2013) *Hum Pathol*. **44**(9):1707-13.
3. Carina, V et Al. (2013) *Thyroid*. **23**(7):829-37.
4. Salmena, L et al. (2011) *Cell*. **146**(3):353-8.

Novel evidences for a role of dopamine as modulator of intestinal motility: a study on mouse distal colon.

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Keywords: **Dopamine; Colonic motility, Enteric Nervous System**

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. It has been classically considered that the pathological hallmarks of PD affect primarily the substantia nigra. Nevertheless, it has become increasingly evident that PD is a multicentric neurodegenerative process that affects several neuronal structures outside the substantia nigra, among which is the enteric nervous system (ENS). Pathological alterations within the ENS could be involved in the gastrointestinal (GI) dysfunction frequently encountered by PD patients. Dopamine (DA) seems to be a major candidate for the impairment of GI function in PD since its levels were found to be decreased in the ascending colon from PD patients.

However, the effective role of DA, and of its receptors, in the modulation of GI functions is far from being clear. Thus, the aim of this study was to explore the role of DA in the GI tract, using as model the mouse distal colon, analyzing, *in vitro*, spontaneous and neurally-evoked mechanical activity of the circular muscle. DA caused a direct inhibitory effect on the colonic spontaneous contractions, antagonized by SCH-23390, D1 receptor antagonist, and by domperidone, D2 receptor antagonist. In addition, DA induced a significant decrease in the amplitude of the neurally-evoked cholinergic contractions, affected by SCH-23390 and by L-NAME, nitric oxide (NO) synthase inhibitor, but not by domperidone. SCH-23390 *per se* increased the amplitude of both spontaneous and neurally-evoked cholinergic contractions. In conclusion, in mouse distal colon, dopamine is a negative modulator of GI motility *via* activation of D1 and D2 receptors. Both receptors are available for pharmacological recruitment, even if only D1-like receptors appear to be preferentially stimulated by endogenous DA. D1 receptors slow down the mouse colonic motility, reducing acetylcholine release from ENS *via* a NO-dependent pathway.

The Sea Urchin Sns5 Chromatin Insulator Settles A Gene Therapy Vector Into An Independent Domain Of Expression In The Vertebrate Genome

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Keywords: **Position effect (PE), lentiviral vector (LV), Chromosome Conformation Capture (3C)**

One critical aspects of introducing a transgene into the eukaryotic genome is the great variability of gene expression due to position effects(1). Chromatin-dependent repressive states could be overcome by incorporation in the transgene of chromatin insulators, functioning to establish domains of expression. We have previously demonstrated that the sea urchin sns5 DNA element has typical features of an insulator: by acting as enhancer blocker, it shields promoters from neighboring regulatory elements, and by acting as barrier it buffers a transgene from the propagation of condensed chromatin(2-4).
We have investigated the use of sns5 in the field of gene therapy. Our preliminary studies shown that the inclusion of sns5 in γ-retroviral vectors allows position-independent expression in erythroid cells. Moreover, transcription factors and histone modifications mark the sns5 chromatin at the integration site(5), suggesting that sns5 displays mechanisms of action common to other well characterized insulators.
Here we show that sns5 increases the likelihood and the expression of a β-globin/lentiviral vector integrated as a single copy in both murine cell clones and in a mouse model of β-thalassemia.
It has been proposed that two copies of insulators may direct the formation of a chromatin loop by interaction among protein complexes assembled on their sequences(6).Intriguingly, by using the 3C technology, we found that sns5-flanked vectors integrated at a single copy in the genome are specifically organized into an independent chromatin structure.
Our findings highlight that sns5 could be a promising tool for improving the performance of vectors in the field of gene therapy.

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2. Palla et al (1997). PNAS USA 94:2272-7
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6. Wallace and Felsenfeld (2007). Curr Op Genet Dev 17:400-7

Biotecnology and Cultural Heritage: bioactive molecules applied in restoration projects

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Keywords: **Biocleaning, Protease, Antimicrobial peptides**.

In the present work bioactive molecules (BM) isolated from marine invertebrate organisms (Anthozoa) are utilize to hydrolyze protein layers (Protease activity) or to control microbial colonization (Antimicrobial activity). Bioactive molecules with high protease activity (BMP) are able to hydrolyze protein layers present on works of art surfaces, acting in a range of temperatures of 4-37°C. This cleaning protocol, safe for both operator and environment, guarantee a selective and, step by step, controllable procedure, respectful of manufact constitutive materials. Enzymatic cleaning was also performed by commercial Protease from *Aspergillus sojae* (Type XIX, Sigma) comparing the hydrolytic activities. Antimicrobial bioactive molecules (BMA1, BMA2) were tested to control bacteria (*Bacillus, Micrococcus*) or fungi (*Aspergillus*, *Penicillium*) growth. These colonies were previously isolated from colonized canvas samples and characterized by an integrated approach (1) based on *in vitro* culture, morphological identifications (OM, SEM) and molecular investigation (PCR and sequence analysis). BMAs were tested in order to define the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal/Fungicidal Concentration (MBC/MFC). BMAs have been tested to control the fungal growth, mimicking the relining of ancient/degraded paintings, on laboratory specimens made with canvas layers and glue paste as binder.
The use of these molecules can be used in innovative protocols, for biocleaning and antimicrobial growth control, according to conservative/restoration procedures safe for both operators and environment (2).

This study was supported by It@cha, "Ricerca e Competitività 2007-2013", PON 01_00625 research project (FP) and by FFR-UNIPA research grant (MC).
(1) Palla, F. (2012) *Science and Conservation for Museum Collections*. Nardini, Firenze, 459-470
(2) Palla F. et al. (2013) *Science and Technology for the Conservation of Cultural Heritage*. Taylor and Francis: London, 279-282

UbcD1 is a Histone H2B Ubiquitin-Conjugating Enzyme Essential for Global Chromatin Structure and Gene Expression Regulation

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Keywords: **Drosophila, H2Bub1, chromatin dynamics**.

Eukaryotic cells have evolved elaborate mechanisms to grant chromatin plasticity and control numerous biological processes, including cell division and differentiation, gene transcription and DNA repair. In contrast to the well-characterized acetylation and methylation, the role of histone ubiquitination in chromatin remodelling remains the least understood despite the long history of its discovery. Interestingly, recent advances highlighted a key role of H2B mono-ubiquitination (H2Bub1) in stem cell differentiation and cancer development. However, the identities of enzymes catalyzing this histone post-translational modification, as well as its effects on global chromatin structure and gene expression, are still fragmentary. *Effete* is an essential gene encoding for the putative E2 ubiquitin-conjugating enzyme UbcD1 playing essential roles in D.melanogaster telomere stability. Nevertheless, the exact molecular mechanism underlying telomere defects observed in flies lacking UbcD1 has not been yet identified. We found that

UbcD1 plays a key role in shaping global chromatin structure. In particular, our *in vivo* and *in vitro* data clearly demonstrate that UbcD1 is important to modulate specifically the mono-ubiquitination of histone H2B. Altered H2Bub1, induced by *effete* loss of function downstream the transcriptional start sites, deeply affects gene expression. Taken together our data identify an unexpected role for UbcD1 in ubiquitination of histone H2B and in regulating global chromatin structure and transcription.

How cancer cells cross lymphatic endothelium?

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The lymphatic microcirculation is a key component of metastatic spread providing a pathway for cancer cell dissemination, and the endothelium seems to enhance or promote the invasiveness of certain cancer cells.
Furthermore, some tumors secrete lymphangiogenic growth factors acting on the lymphatic vasculature to facilitate metastasis. This study, based on 875 ultrastructural serial sections and 9 three-dimensional reconstructions of 28 initial lymphatics, clarify the transmigration mechanism of cancer cells from the extravascular matrix to the lumen of initial lymphatics. The lymphatic identification and distribution were assessed by transmission electron microscopy and immunohistochemistry (D2-40 and LYVE-1 markers) in experimental murine tumor mass (T84 adenocarcinoma, B16 melanoma, and transgenic prostate adenocarcinoma).
The initial lymphatics were detectable in peritumoral connective tissue showing an endothelium lacking of continuous basal membrane. Twenty-nine invasive phenotype cancer cells were detected to migrate from the extravascular matrix of peritumoral connective tissue towards the initial lymphatic taking firm adhesion with the abluminal endothelial wall. The ultrastructural feature analysis and their reconstruction showed a cancer cell passage through the *interdigitating* and *overlapping* interendothelial contacts of the lymphatic vessels, without evidences of endothelial barrier degradation indicating the plasticity of endothelial cells.
This study provide concrete contribution on the mechanism underlying the interactions between cancer cell and lymphatic endothelium, demonstrating a cancer cell transmigratory pathway. The discovery of the molecular mechanisms controlling these interactions could lead to new therapeutic strategies to reduce metastatic diffusion.

ACKNOWLEDGEMENT: I would like to thank Prof. Giulio Ghersi for his supervision.

A proteomic signature for breast cancer patients stratification

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Keywords: **Breast cancer, Proteomics, Signature**.

Breast cancer is a complex and heterogeneous disease with respect to histological grading, cellular origin, mutations, metastatic potential, disease progression, therapeutic response and clinical outcome [1,2].
This study represents the prosecution and updating of a research for the detection of a proteomic signature associated with breast cancer, useful for the molecular-classification of patients. To achieve this goal, we have used the two dimensional gel electrophoresis (2D-IPG) procedure, coupled with mass spectrometry (MS) for protein identification. The investigation was carried out on a large set of surgical sample of breast cancer tissues (n =76), diagnosed as ductal infiltrating carcinomas. The identified proteins were grouped into functional categories by a gene ontology classification.
Interestingly, three different driving-clusters (programmed cell death, glycolysis and cell motility) were extracted from the combination of experimental proteomic with bioinformatic. Each proteomic cluster, was expressed in a variable percentage among patients. The expression pattern of the protein clusters involved

in the apoptotic and glycolytic pathways were well represented in all patients, strongly suggesting that their expression is essential for the primary tumor growth. Conversely the cluster of cell motility proteins was irregularly expressed among patients, indicating this cluster as a possible marker for tumor metastasis, and suggests the possibility of using it as prognostic factors for breast cancer progression. Hierarchical clustering strengthen this hypothesis and showed the possibility to segregate patients in different class according to the probability of disease progression.

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The synergistic effect exerted by the HDAC inhibitor SAHA and the sesquiterpene lactone parthenolide on triple negative breast cancer cells.

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Keywords: **triple negative breast cancer cells, parthenolide, histone deacetylates inhibitor, apoptosis**

Triple-negative breast cancer (TNBC) is a subtype of breast cancer, insensitive to endocrine therapy. Chemotherapy is the main form of treatment, but is accompanied by a high rate of recidivism. The sesquiterpene lactone Parthenolide (PN) exerts a cytotoxic effect on MDA-MB231 cells, a TNBC cell line (1), but was ineffective at low doses (2-5μM). This represents an obstacle for a therapeutic utilization of PN. We supposed, in line with other authors (2), that PN causes a protective response, which at low doses prevails on the cytotoxic effect. With the aim of inhibiting this protective effect we have shown that pre-treatment of MDA-MB231 cells with SAHA (2-5μM), an histone deacetylates inhibitor, synergistically sensitizes the cells to the cytotoxic effect of PN, also at low doses of this compound.

SAHA/PN combination induced hyperacetylation of histones H3 and H4 and hypomethylation of DNA. These changes cause epigenetic effects, which can be responsible for the increased expression of tumour suppressors p21 and p27 and decreased levels of Bcl2 and p65, a component of NFkB.

Moreover SAHA alone induced ROS generation as well as autophagy, which favours cell survival, and apoptosis. The addition of PN (8μM) to SAHA reduced production of ROS and autophagy, while increased the apoptotic process.

Interestingly PN activates Akt, mTOR, phospho-p70S6kinase and ULK1/2, a factor that inhibits autophagy. In addition PN caused nuclear accumulation of Nrf2 with stimulates antioxidant genes. SAHA prevented these effects.

In conclusion SAHA/PN stimulated cytotoxicity through many mechanisms: (i) induces epigenetic events with changes in gene expression, (ii) PN prevents SAHA effect on autophagy and (iii) SAHA suppresses the protective response exerted by PN through inactivation of m-TOR. Taken together our results suggest that combination SAHA/PN can be a candidate for TNBC therapy.

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The growth inhibition of $(Bu_3Sn)_4TPPS$ and $(Bu_2Sn)_2TPPS$ treated human melanoma cells is associated to decrease of adhesion receptors expression.

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Melanoma is the cancer with the higher incidence in western populations and unfortunately, despite the increase of early diagnosis, the mortality of melanoma-affected patients is still raising. Melanoma is notoriously resistant to all current cancer therapy notwithstanding several studies were performed to identify new and more effectives chemotherapeutic agents. Therefore, in the aim to identify new potential anti-tumour drugs for this aggressive type of cancer, we studied the $(Bu_3Sn)_4TPPS$ and $(Bu_2Sn)_2TPPS$ organotin-meso-tetra(4 sulfonatophenyl) porphine derivatives, and we showed that these compounds induce the death for apoptosis of melanoma cells [1]. Moreover, we identified the concentrations of $(Bu_2Sn)_2TPPS$ and $(Bu_3Sn)_4TPPS$ sufficient to significantly reduce the melanoma cells growth [2]. In order to study the role of organometallic complexes on the invasion and metastasis of melanoma, we treated the A375 and HT144 melanoma cells with the concentrations of $(Bu_2Sn)_2TPPS$ and $(Bu_3Sn)_4TPPS$ previously identified. The alteration of adhesion receptors expression is related to metastatic progression of melanoma through the deregulation of adhesive functions and the subsequent detachment of tumour cells from the primary tumour. Interestingly, in both treated cell lines we showed a cell morphology alteration, a reduced expression of adhesion receptors such as Integrins $\beta1$ and $\beta3$, MCAM and ICAM, a decrease of Epithelial-Mesenchymal-Transition (EMT) proteins expression such as β-catenin and N-cadherin and the decrease of gelatinolytic activity of secreted metalloproteinases (MMP2 and MMP9). The results obtained, suggested a non secondary role for $(Bu_2Sn)_2TPPS$ and $(Bu_3Sn)_4TPPS$ in the regression of the melanoma invasive-metastatic state.

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MicroRNA-29b-1 is involved in self-renewal and fate decisions of human osteosarcoma 3AB-OS cancer stem cells

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Key words: **osteosarcoma, cancer stem cells, microRNA**,

Emerging evidence suggests that treatments targeting cancer stem cells (CSCs) within a tumor can halt cancer and improve patient survival. Moreover, identification of CSC-related MicroRNAs (miRNAs) would provide information for a better understanding of CSCs. miR-29 family is a class of miRNAs aberrantly expressed in multiple cancers. They are frequently down-regulated in osteosarcoma (OS), the most common form of childhood cancer with a potent metastasizing potential. 3AB-OS CSC, a human pluripotent CSC line by us produced from the human osteosarcoma MG63 cells (1) is a useful model to study CSC origin and roles (2). Previously, we have shown that in 3AB-OS CSCs miR-29b is potently down-regulated (2). Here, after stable transfection of 3AB-OS cells with miR-29b-1, we investigated its role in regulating cell proliferation, sarcosphere-forming ability, clonogenic growth, chemosensitivity, migration and invasive ability of 3AB-OS CSCs, *in vitro*. We found that miR-29b-1 overexpression consistently reduced both, 3AB-OS CSCs growth in

two- and three-dimensional culture systems and their sarcosphere- and colony- forming ability. It also sensitized 3AB-OS cells to chemotherapeutic drug-induced apoptosis. Using publicly available databases, we proceeded to identify potential miR-29b target genes, known to play a role in the above reported functions. Among these targets we analyzed CD133, N-Myc, CCND2, E2F1 and E2F2, Bcl-2 and IAP-2. We even analyzed the most important stemness markers as Oct3/4, Sox2 and Nanog. Real-time RT-PCR and western-blot analyses showed that miR-29b-1 negatively regulated the expression of these markers. Overall, the results show that miR-29b-1 suppresses stemness properties of 3AB-OS CSCs and suggest that developing miR-29b-1 as a novel therapeutic agent might offer benefits for OS treatment.

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Role of Nupr1/p8 in hepatocellular carcinoma: implications in cell growth control and response to treatment

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Nupr1/p8 is a stress-inducible protein over-expressed in different malignancies which can act both as a tumor inducer or suppressor. The role of Nupr1/p8 in hepatocellular carcinoma (HCC) has not been fully elucidated. Here, we examined Nupr1/p8 expression in HCC and in healthy liver tissues. Immunohystochemistry and qPCR analyses revelead higher expression level of Nupr1/p8 (both protein and mRNA) in HCC tissues than in normal liver tissues. Then, we used well-established in vitro models of human HCC to investigate the role of Nupr1/p8 in hepatocarcinogenesis and in chemoresistance. Transient siRNA-mediated Nupr1/p8 knockdown in HCC cells led to sharp decrease of HCC cell growth, migration and colony formation and increased HCC cell sensitivity both to doxorubicin and sorafenib. Initial gene expression analysis by qPCR showed that Nupr1/p8 suppression altered the expression of several genes involved in a pro-survival pathway, such as *RelB* and *IER3*, and genes involved in ER stress response, such as *TRB3*. Consecutively, shRNA-mediated Nupr1/p8 knockdown was used for the generation of stable HCC Nupr1/p8$^{-/-}$ clones, which were injected in nude mice to study *in vivo* the role of Nupr1/p8 in HCC tumorigenesis. We found that Nupr1/p8$^{-/-}$ cells were unable to form tumor in nude mice, suggesting that Nupr1/p8 is essential for tumor growth *in vivo*. To clarify the molecular mechanisms of Nupr1/p8 action in HCC, we studied global gene expression changes in Nupr1/p8$^{-/-}$ HCC cells, using microarray analysis. Nupr1/p8 depletion altered expression levels of 446 genes involved in a variety of cellular processes, including not only cell death and proliferation, but also lipid metabolism. Preliminary results showed that Nupr1/p8 is overexpressed in steatotic liver tissues and its depletion reduced lipid droplets accumulation in Nupr1/p8$^{-/-}$ cells, after oleic acid treatment. Thus, our data suggest that Nupr1/p8 represents a new therapeutic target for treatment of HCC, which could also play a key role in liver steatogenesis.

Metabolic Pathways in *Microbispora sp.* ATCC-PTA 5024, Producer of NAI-107 Lantibiotic

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Key words: **Lantibiotic; Proteomics; Actinomycete**

The actinomycete *Microbispora sp*. ATCC-PTA-5024 produces the lantibiotic NAI-107 (1) a promising drug to treat multidrug-resistant pathogen infections (2). *Microbispora* is a poorly characterized and this limited knowledge is detrimental to set-up NAI-107 production processes to efficiently deliver high-quality compound. High throughput techniques, like proteomics, may give insights on strain molecular physiology and biochemical capability and, above all, on metabolic pathways and regulatory mechanisms thereof associated with antibiotic production (3).
Thus *Microbispora* differential proteomic analyses were comparatively carried-out on wild type, null and super-producer strains by mean of 2-D-Differential Gel Electrophoresis and mass spectrometry procedures.
This study revealed differential regulation of pleiotropic regulators, stress response factors and proteins involved in many cell processes and metabolic pathways associated with NAI-107 production on-set and maintenance. In particular, proteins involved in molecular processes like amino sugar, nitrogen, phosphate and sulphur metabolism, oxidative stress and antibiotic biosynthesis and resistance are positively correlated while proteins involved in glycolysis, amino acid and nucleotide metabolism are negatively associated to NAI-107. Therefore, these data coupled to gene ontology, revealed a comprehensive set of differentially regulated proteins which may play roles as trigger or sustaining or response factors in NAI-107 production.
Altogether this information may be used as a knowledge background to rationally improve NAI-107 production by *Microbispora* fermentation optimization or for strain improvement by genetic engineering on targeted genes.

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Chromatin remodelers, nucleoplasm compartment and proteinopathies

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Recent advances in the field of transcriptome exploration have revealed novel sets of new ncRNAs like the long non-coding RNAs, which seem to be key components of epigenetic regulatory networks. Indeed, recent studies have shown that lncRNAs regulate the gene expression by chromatin remodelling, transcription, splicing and RNA decay control, enhancer function, and epigenetic regulation. An emerging theme from multiple model systems is that lncRNAs form extensive networks of ribonucleoprotein (RNP) complexes with numerous chromatin regulators and then target these enzymatic activities to appropriate locations in the genome. Using D. melanogaster as model system, I recently found a functional interaction between ISWI, the catalytic subunit of several ATP-dependent chromatin-remodeling complexes, and the lncRNA hsr-omega (hsrω). In Drosophila the nucleus-limited hsrω-n transcript is dynamically associated with several different

heterogeneous nuclear ribonucleoprotein (hnRNPs) in the nucleus to organize the omega speckles. Omega speckles, play essential roles in the regulation of RNA processing reactions and their alteration could promote aberrant expression of hnRNPs with severe change in various mRNA expression and processing of their targets genes. Indeed, emerging neurodegenerative diseases as proteinopathies, seem to be caused by alteration in hnRNPs nucleus amount and localization. I have evidence that the hnRNP TBPH, the Drosophila homolog of human TAR DNA binding protein (TDP-43 or TARDBP), the major protein present in cytoplasmic inclusions in neurodiseases, is a component of the omega speckles. Interestingly, I have data strongly suggesting that ISWI is involved in the regulation of correct localization and functioning of TBPH protein. I report preliminary results showing in D. melanogaster the role of of chromatin remodelers in nucleoplasm compartment organization and their possible role in proteinopathies onset and development.

A molecular strategy to cope with serpinophaties.

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Keywords: **protein self-assembly, serpin, conformational diseases, drug discovery**

The serpinophathies are a group of heterogeneous pathologies caused by a common molecular mechanism: the polymerization of a mutant member of the serpin (SERine Protease INhibitor) superfamily of proteins. The diseases are characterized by a gain-of-toxic-function phenotype, due to the presence of polymer inclusions within the cells of synthesis, and a loss-of-function phenotype, due to the concomitant lack of the active serpin in the extracellular place of action. The most common and best-known serpinopathy is the α_1-antitrypsin deficiency, caused by mutations in the prototypical serpin α_1-antitrypsin (AAT), and characterized by lung emphysema, due to uncontrolled elastase activity in the lungs, and liver disease, due to retention of polymerized AAT within the hepatocytes. Other serpinopathies taken into account in our studies are the Familial Encephalopathy with Neuroserpin Inclusion Bodies caused by mutations in neuroserpin (NS), a neuronal inhibitor of tissue plasminogen activator, and the Hereditary Angio-Edema due to the lack of C1-inhibitor, an acute phase protein capable to inhibit the esterase activity of the first component of the complement system.
We exploit different biophysical techniques (including Optical Spectroscopies, Small Angle X-ray Scattering, Dynamic Light Scattering, Atomic Force Spectroscopy, Liquid Chromatography, Molecular Dynamics) to understand the conformation of serpin dysfunctional states and the controversial structure of serpin polymers. Our results highlighted the mechanism of polymerization along with the main structural features of antitrypsin and neuroserpin polymers. Such structural achievements are used as a starting point for a rational drug discovery. The screening of known lead compounds and their docking in silico allowed us to select a few small molecules, whose capability to interfere with NS polymerization is tested in vitro.

Clever pH-Sensitive Drug-polymer Conjugates For Targeted Cancer Therapy

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Keywords: **prodrugs, pH sensitive, drug delivery, targeted cancer therapy, polyaminoacids, polysaccharides**

Macromolecular prodrugs are being used as main delivery model in nanomedicine so as to successfully release anticancer drugs with very low bioavailability and, consequently, poor effectiveness in vivo (1). Many research groups have attempted to design systems with outstanding and localized anticancer properties, avoiding by-effects onto healthy tissues. However, so far, those proved capable of reduce tumor mass in mouse model have not shown the same activity in humans, thus remarking that different approaches in terms of drug release mechanisms should be employed (2). Perhaps, this behavior might be related to retro-

diffusion of drugs ones quickly released inside cells, mainly assisted by the pump efflux of tumor cells. Here, the design of pH sensitive macromolecular prodrugs was taken into account in order to synthesize highly water soluble drug-polymer conjugates, though carrying high amount of doxorubicin as drug model, able to release their payload by pH-sensitive cleavage mechanism triggered at lysosomal pH (5.5). This was accomplished by coupling doxorubicin though cis-citraconic acid to multifunctional polymers, that is α,β-poly(N-hydroxyethyl)-D,L-aspartamide (PHEA) and inulin (INU), endowed with high water solubility and biocompatibility. The cis-citraconic acid, used as spacer, was selected owing to its ability to form amide groups cleavable in lysosomal and mitochondrial media with a degradation rate suitable to elude the usual multidrug resistance mechanisms, thus provoking cell apoptosis or necrosis. Additionally, the two polymer conjugates were equipped with pendant alkynes to orthogonally bond thiolated targeting agents (thio-RGD) via thiol-yne click-chemistry. The ability of the drug-polymer conjugates was established in vitro on human colon tumor (HCT116) and human bronchial epithelial (16HBE) cell lines.

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Enzymes involved in Anderson-Fabry Disease

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Fabry Disease, α-GAL A, alpha-NAGAL

Since 2005 our group has studied enzymatic and genetic alterations in Anderson-Fabry disease (FD). FD is a hereditary dysfunction of the metabolism of glycosphingolipids, mostly globotriaosylceramide (GB3).
FD has clinical manifestations and variable course that lead to the death of the patient within the fifth decade of life, if not diagnosed promptly.
FD is a lysosomal enzymopathy X-linked caused by mutations occurring in the GLA gene which encodes for the enzyme α-galactosidase A (α -GAL).
Diagnosis is proposed basing on clinical data and family medical history, then it is confirmed by genetic and biochemical tests. These tests are the direct sequencing of the GLA gene and the determination of the enzymatic activity of α-GAL A, which can be low or zero.
In the last years, tests were performed on more than 6000 subjects, with signs and symptoms referable to FD, and on 2436 healthy control subjects.
Clinical diagnosis of FD was confirmed in 112 out of all the studied subjects, who had mutations in the exons of the GLA gene related to the deficit of the enzymatic activity of α-GAL A.
The study of the mutations and the genotype-phenotype correlation revealed that some patients with an exonic mutation and the enzymatic deficit, didn't show a severe and expected clinical picture, typical of the disease, but they manifested faint symptoms and, moreover, some of this patients were old-aged, feature conflicting with FD.
Therefore, we reckon that one or more mechanisms can provide for α-GAL A deficiency.
One of our project aims at studying these replacement and/or offsetting mechanisms of α-GAL A deficiency. We hypothesized the activation of an alternative pathway for the metabolism of GB3 that involves the isoenzyme of α-GAL A, the so-called α-GAL B or α-NAGAL.
α-GAL B is structurally similar to α-GAL A and it hydrolyzes GB3 when its concentration in lysosomes is elevated. Therefore, enzymatic and genetic analyses of α-GAL B will be also performed on the patients described above.

Thanatos associated protein 11 (THAP11) modulates expression of c-MYC by binding the HB2.8 enhancer blocker element

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Keywords: **c-MYC, THAP11, chromosome conformation capture (3C)**

C-MYC is one of the most frequently deregulated genes in human tumors. A detailed understanding of c-MYC transcriptional control is essential to better understand the molecular aspects of its several fuctions.
We have characterized an enhancer blocker element (HB2.8) located 32Kb downstream of the c-MYC gene and, by using different methods (EMSA assay, 2D-IPG and MALDI analysis), we identified several proteins able to bind this region.
Among these proteins, we focalized our attention on THAP11 (Thanatos-associated proteins 11), a member of THAP proteins family, which is involved in cell proliferation, apoptosis, chromatin modification and transcriptional regulation[1]. THAP11 is ubiquitously expressed and frequently downregulated in several human tumor tissue[2].
To confirm that THAP11 directly binds the HB2.8 element *in vivo*, we performed Chromatin Immunoprecipitation (ChIP) in HELA and U937 cells. To further evaluate the role of THAP11 on c-MYC gene transcription, we investigated the binding of THAP11 to the c-MYC P2 promoter and HB2.8 element. To this end, we compared proliferating and differenziated U937 cells finding a significant enrichment of THAP11 binding in differentiated cells, which correlates with downregulation of c-MYC gene.
We hypothesized that c-MYC expression may be regulated by the interaction of the P2 promoter and the HB2.8 enhancer blocker element, mediated by THAP11. To confirm this hypothesis, we investigated the genomic organization around the c-MYC locus by using chromosome conformation capture (3C) and a qPCR based analysis. The 3C experiments confirmed an enrichment in the interaction frequency between the c-Myc P2 promoter and HB2.8 in U937 differentiated cells compared to U937 proliferanting cells, suggesting that THAP11 may be involved in c-MYC locus chromatin remodeling.

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Diet-Induced Obesity: A Risk Factor for Alzheimer's disease

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Keywords: **Alzheimer's disease, Obesity, Insulin Resistance.**

Alzheimer's disease is the leading cause of dementia and the most prevalent neurodegenerative disease (1). It is an aging-related multi-factorial disorder (2) in which obesity has been recognized as an important player in the pathogenesis of this type of dementia, independently or not of insulin resistance. Insulin is involved in the modulation of synaptic plasticity and learning memory. Thus, irregularities in the insulin signaling pathway might contribute to impairment of memory function, comparable to those observed in patients with AD (3). Insulin resistance could initiate a neurodegenerative cascade that increased oxidative stress, neuro-inflammation and impaired neuronal survival (4). To understand the mechanisms that link Obesity and AD we used a high fat diet-induced model of obesity with the aim to determine whether changes in protein involved in AD, such as APP, expression occurred in relationship with an insulin resistance and oxidative stress status.

C57BL6/J mice were fed a high fat diet (HFD) or a standard diet (STD) for fourteen weeks. Compared with age-matched control animals, HFD mice were insulin resistance. Indeed HFD mice displayed significantly increased levels of blood glucose after intraperitoneal exogenous administration of insulin compared with STD mice. Western blot and array profiling, of brains of obese and control mice, showed change in expression level of proteins and genes, involved in insulin resistance. Western blot analysis and immunohistochemistry showed changing in expression of proteins related to AD as APP, Presenilin1, BACE and Gsk3β. Moreover, an increase of ERK and iNOS, markers of oxidative stress, together to a decrease of Heme Oxigenase, an antioxidant protein, was detected. The obtained results strongly suggest the presence of a link among obesity, insulin resistance and AD.

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NANOGELS AS USEFUL TOOL FOR ALZHEIMER'S DISEASE THERAPY

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Keywords: **Alzheimer's disease, Nanogels, insulin**

The importance of insulin in glucose regulation is well known. However, its relevance is not limited to glucose metabolism. It has been demonstrated that insulin signaling is involved in brain cognitive functions and their dysfunction in ageing brain degeneration. Thus, insulin administration could be a potential therapeutic agent for neurodegenerative diseases such as Alzheimer's disease (AD). For the purpose of crossing the blood-brain-barrier (BBB), nanocarriers can be a valuable help. Nanogels (NGs) have a great potential in the development of "smart" nanocarriers for (bio)molecular drugs and contrast agent for bioimaging. They are formed by physically or chemically crosslinked polymer networks, characterized by a large and flexible surface available for multivalent bioconjugations. NGs can be produced with high yields and through-puts by pulsed electron-beam irradiation of dilute aqueous solutions of water-soluble biocompatible polymers. In this work, a carboxyl functionalized nanogel system (NG), generated by pulsed e-beam irradiation of a semi-dilute poly(N-vinyl pyrrolidone) (PVP) aqueous solution in the presence of acrylic acid, with an average diameter in the 60-70 nm range (PDI<0.3) was used as a substrate to generate chemically stable insulin-grafted PVP NGs. In particular, grafting was carried out using human insulin without (PVP-g-insulin) or with fluorescein isothiocyanate labeling (PVP-g-insulin-FITC). The hydrodynamic dimensions of NGs before and after grafting ("naked NGs" and "grafted NGs") were investigated by Dynamic Light Scattering. The PVP-g-insulin-FITC system was used in order to both quantify the conjugation degree of insulin to the nanoparticles by UV-vis spectroscopy and to study NGs localization in cell cultures. Different conjugation degrees were obtained by varying the reaction conditions. Biocompatibility tests of naked and insulin-grafted NGs were performed on neuroblastoma LAN5 cells by MTS assay. Co-localization of PVP-g-insulin-FITC NGs with activated insulin receptor was detected by immunohistochemistry technique and microscopical observations. Finally, the biological effect of insulin-grafted NGs was verified by activation of Akt and FOXO3a, two molecules involved in insulin signaling.

Streptomyces coelicolor: DNA methylation and differentiation

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Keywords: **DNA methylation, *Streptomyces*, morphological and physiological differentiation**.

DNA methylation is an epigenetic modification regulating many aspects of biological processes. DNA cytosine methylation plays mainly a regulatory role in chromatin organization, genome maintenance and gene expression in eukaryotes, while its role has not been deeply investigated in prokaryotes (1-2). Differently, DNA adenine methylation regulates chromosome replication, DNA repair, transposition of insertion elements in prokaryotes, while it is supposed to have exclusively a role in regulating gene expression and DNA replication in mitochondria.
Streptomyces coelicolor is a soil-dwelling Gram-positive bacterium that exhibits a complex life cycle, with three different cell types (unigenomic spores, aerial and vegetative hyphae) and two events of programmed cell death (3), and produces three antibiotics.
The aim of this project is to correlate DNA methylation with morphological and physiological differentiation of *S. coelicolor*.
Dot blot analysis revealed that the global level of methylated cytosines and adenines changes during growth in liquid medium. The characterization of cytosine methylome, by Bisulphite-sequencing, revealed that 30% of *S. coelicolor* genes contain a AAGCC^{m}CG or TGGC^{m}CGGC motif in their upstream regions. Among these, genes related to differentiation, DNA repair and condensation were found. Treatment with 5-aza-2'-deoxycytidine, a hypomethylating agent, showed that DNA methylation influences growth and antibiotic production both in liquid and on solid culture.
These results suggest a role for DNA cytosine methylation in morphological and physiological differentiation of *S. coelicolor*. Further experiments are ongoing to demonstrate if it has a role in regulating gene expression and/or in activating cell death events.

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Protein diffusion in ovo.

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Keywords: **Diffusion, Molecular Crowding, Fluorescence Correlation Spectroscopy, Dynamic Light Scattering**.

Molecular crowding at the cellular level is capable of changing the properties of the macromolecules involved in disparate biological processes, and it is thought to affect differently small and large molecules.
The hen egg albumen has a high protein content (~10%), mainly ovalbumin, and it is a simple and yet rich model of a crowded biological environment, even if moderately crowded, with respect to other cellular environments.
We used two techniques for studying different aspects of the molecular motion in this system: Fluorescence Correlation Spectroscopy (FCS), that reveals the self-diffusion of particles labeled with a fluorescent probe, and Dynamic Light Scattering (DLS), that measures the collective diffusion of all particles, as affected by intermolecular interactions.
The results on the albumen samples have been compared with those on a more controlled system containing ovalbumin as a single component. In the latter case it was also possible to explore a range of protein concentrations higher than that found in the albumen.
The slowing down of ovalbumin molecules in the albumen is comparable to that observed in a pure

ovalbumin solution at a concentration of ca. 80mg/ml.
Moreover in this range of concentrations the hydrodynamic radius increases by a factor of 1.7 with a limited degree of nonlinearity, which turns evident for concentrations larger than 150mg/ml.
The albumen was also investigated with static and dynamic light scattering, revealing the presence of two relaxation processes, one related to the diffusion of the monomeric protein molecules and a slower one related to the motion of sub-micrometer elongated objects.

RNA binding proteins in brain cells differentiation

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Keywords: **RNA Binding Proteins, brain cells, Histone variants**

The complex interplay of post-transcriptional regulatory mechanisms mediated by RNA-binding proteins (RBPs) at different steps of RNA metabolism is pivotal for the development of the nervous system and the maintenance of adult brain activities, and depends on RBPs (1). RBPs are able to regulate translation, stability and subcellular localization of mRNAs. Two proteins the expression of which is mainly post-transcriptional in developing mammalian brain are the histone variants H1° and H3.3 (2-3). We isolated two RBPs able to bind these brain specific histone variants: PIPPin and LPI (long Pep-19 isoform) (4-5-6).
Searching for components of a hypothetical ribonucleoprotein particle, possibly involved in histone mRNAs metabolism, we evidenced by immunoprecipitation a set of proteins interacting with each other. These proteins were isolated from brain cell nuclei by a chromatographic approach and identified by mass spectrometry; as demonstrated also by western analysis, PIPPin is present among them (7). These results are in agreement with the idea that post-transcriptional regulation of H1° and H3.3 histone expression depends on a group of proteins which are probably part of a specific ribonucleoprotein particle that contain also PIPPin. Such particle should contain, together with specific proteins, also proteins which are known to bind other mRNAs (7).
In addition, by Bio-Layer Interferometry (BLI), we confirmed the ability of the PIPPin protein to bind both M4 and R4 regions of the H3.3 mRNA, as well as d (and in particular SH1) region of H1° mRNA, and we also calculated the KDs of these interactions.

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Hsp60 and GroEL Chaperonins: Thermodynamic Characterization on Self-Assembly and Structural Stability Studied by Nano DSC and Nano ITC

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Molecular chaperones bind to a large variety of different protein folding intermediates to prevent their non-specific aggregation and facilitate protein folding. Folding chaperones, e.g. Heat shock proteins, undergo large conformational rearrangements (driven by ATP-binding, hydrolysis and co-chaperones) that modulate

client–protein interactions. Some of these conformational changes are associated with Intrinsically Disordered Regions [1]. Hsp60 assists the correct folding of mitochondrial proteins and plays a role in cytoprotection against cell stressors. Despite a plethora of studies on its bacterial homologs GroEL, key questions on Hsp60 structure-functions are still unanswered. There are evidence that Hsp60 exists in solution in dynamic equilibrium between monomers, heptameric single rings and double ringed tetradecamers. We use ITC-dilution and Nano-DSC to probe the dissociation equilibrium and thermal stability of Hsp60 and GroEL [2]. Results indicate that Hsp60 exists in a dynamic equilibrium between monomeric and heptameric form, even in the absence of ATP and the 10kDa co-chaperonin [3]. The two proteins unfold with different melting temperatures (T_m) and calorimetric enthalpy changes (ΔH_{cal}). In the case of Hsp60, the transition peak is skewed towards the low temperature side as expected for a transition coupled to dissociation. Experiments at different protein concentration confirm that the unfolding is coupled to the dissociation of the oligomeric protein.

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The Interaction of Small Molecules with Biomolecules

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keyword: **computational chemistry, DNA, host-guest interactions, metal complexes**

The binding of small molecules with biological targets is associated to interesting chemical and biological properties of the resulting supramolecular systems. We have recently reported on the synthesis and characterization of cationic first row transition metal complexes and the study of their DNA binding properties, in aqueous solutions at neutral pH, essentially investigated by viscosimetry and spectroscopic techniques such as circular dichroism, absorption and fluorescence in the UV-visible wavelength range. Of course, such procedure cannot furnish atomic level details of the molecule-DNA interaction. Computational Chemistry may provide support for the interpretation of experimental data on an atomistic level (Fig.1). For example, we have recently shown that Molecular Dynamics (MD) simulations, followed by quantum mechanics/molecular mechanics (QM/MM) calculation, provided detailed structural informations and binding energies of the complexes between nickel(II), copper(II), zinc(II) metallointercalators with nucleic acids in the canonical B conformation [1]. We are presently applying such complementary experimental and computational approach to the interaction of small molecules with G-quadruplex (G4) DNA. The latter is a non-canonical conformation recently observed in human cells [2], and it has been proposed as a target for a novel class of anticancer drugs. Recently we have performed MD simulation to have an insight into the molecular recognition process of small organic ligands and other biological targets, such as mRNA and proteins [3].

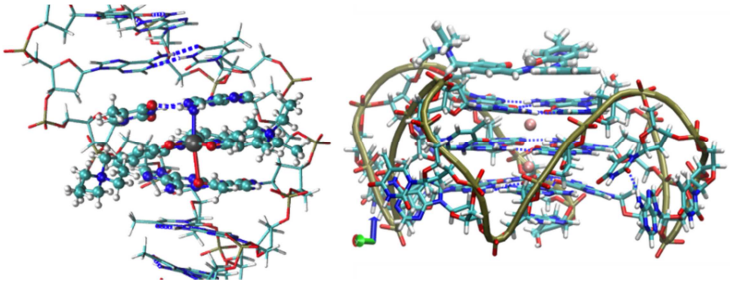

Figure 1: The interaction of a zinc(II) complex with duplex (left) and quadruplex (right) DNA.

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Misdiagnosis and new diagnostic tools in Fabry Disease

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Fabry Disease, Multiple Sclerosis, microRNAs

Fabry Disease (FD) is a hereditary, X-linked, progressive and multisystemic lysosomal storage disease, featuring variable course and clinical manifestations. It is a metabolic disorder caused by the functional deficit of the enzyme α-galactosidase A (α–GAL A). This deficit is responsible for the alteration of the metabolism of some glycosphingolipids and precisely globotriaosylceramide (GB3), which builds up in lysosomes of different cellular types, particularly in vascular endothelium cells. FD is an X-linked lysosomal enzymopathy caused by mutations in the GLA gene, encoding for α–GAL A. It is considered a rare disorder with an estimated incidence of 1:40.000, but actually data in literature show that FD is often seen and rarely diagnosed.
Since FD clinical manifestations overlap to clinical manifestations of other diseases, patients affected by FD are often first diagnosed as affected by common pathologies, different from FD. Among these, Multiple Sclerosis (MS) shows neurological signs and symptoms similar to the ones of FD and it is the first diagnostic hypothesis in 5% of FD patients.
Therefore, we are performing genetic and enzymatic tests in patients with diagnosis of ambiguous MS, in order to identify unacknowledged patients affected by FD.
Moreover, since 98% of subjects with systemic symptoms does not show any mutation occurring in the exons of the GLA gene, more innovative diagnostic tools are required to make a more reliable diagnosis. Among these, microRNAs, currently used to diagnose many pathologies, could be the new era of biomarkers for FD.
Diagnosis of FD needs to be timely in order to start the enzyme replacement therapy (ERT) as early as possible; ERT limits or stops symptoms progression, therefore improving considerably quality of life.

Abstract Poster:

Polyvinylpyrrolidone nanogels for gene delivery studies

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Keywords: **polyvinylpyrrolidone nanogels, e-beam, oligonucleotides**.

A new family of nanogels (NGs) are synthetized by high-energy radiation (e-beam irradiation), starting from a dilute aqueous solution of a commercial polyvinylpyrrolidone (PVP), that can be simultaneously grafted with a variety of functional monomers.The resulting NGs are formulated with appropriate size,shape,surface properties and specific chemical functionality. After single step radiation synthesis, without organic solvents, toxic initiators or catalysts and surfactants, expensive purification procedures are not required and simultaneously, nanogels results sterile. These are essential properties for biomedical applications(2). *In vitro* cells studies demonstrated their biocompatibility, in fact they easily can bypass the cell plasma membrane without inducing cell toxicity phenomena. The nanoparticles are functionalized with amino and carboxyl groups to attach ligands for specific cell receptor (targeting activities) or siRNA for gene therapy applications(1).
In this view, PVP-based nanogels, functionalized with carboxyl groups, are linked to the 3'-amino modified oligonucleotides, made fluorescent by the presence of the FAM dye on the 5'-end. The conjugation degree of the oligonucleotides-nanogels complex was evaluated by spectrofluorimetric readings. Moreover, annealing studies were made to see if the sequence was available despite the conjugation to the nanogels, using a reverse oligonucleotide carrying a fluorescence quencher. The specific pairing of bases was also tested by enzymatic digestion, using a restriction enzyme, PST I(3). Once established the ideal conditions for the nanogels conjugation with nucleic acids, and tested their accessibility and stability, NGs have been linked to amino-modified specific siRNA, for future studies of gene delivery.

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P. lividus oogenesis and early development require autophagy

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It is well known that autophagy is a major intracellular pathway for the degradation and recycling of cytosolic components, in both basal and stress conditions.
We have recently demonstrated the activation of autophagy in *P. lividus* embryos: at high levels, after a stress induced by cadmium, and at basal levels, during the physiological development (1) (2).
Here we report our recent data about autophagy during oogenesis and segmentation. In order to detect autophagolysosomes (AVOs) and autolysosomes, we respectively performed incubation *in vivo* with acridine orange (AO), and in situ analysis by immunofluorescence (IF) with the anti-LC3 antibody (autophagy marker). All observations were carried out through Confocal Laser Scanning Microscopy.
In this way, we studied the trend of autophagy during oocytes maturation, after fertilization and during early developmental stages. Results showed that, in all cases, the autophagic markers have specific localizations.
Furthermore, we observed that the treatment with bafilomycin A1 (an inhibitor of autophagy) for 2 hours, after fertilization, generates developmental delays and morphological anomalies, especially visible after 24 hours, demonstrating that autophagy is essential during the early developmental stages.
These data showed the occurrence of autophagy in oocytes and during segmentation: in the former case probably for the recycling of cellular components that accumulate during oogenesis and for the elimination

of the germinal vesicle, in the latter case, perhaps for the elimination of certain cellular components that are no longer required in those developmental stages.

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Encapsulation of a Multikinase Inhibitor for the Treatment of Human Hepatocellular Carcinoma

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Keywords: **multikinase inhibitor, nanostructured lipid carriers, clonogenic assay**

Sorafenib is a multikinase inhibitor approved in the EU for the treatment of patients with advanced hepatocellular carcinoma. It inhibits cell surface tyrosine kinase receptors (VEGFR and PDGFR-β) and downstream intracellular serine/threonine kinases (e.g. Raf-1, wild-type B-Raf and mutant B-Raf), which are involved in tumor cell proliferation and tumor angiogenesis. In vitro sorafenib shows dose-dependent antitumor activity determining induction of apoptosis and inhibition of cell proliferation. However, the poor aqueous solubility and undesirable side effects (diarrhoea and hand-foot skin reaction, alopecia, anorexia, weight loss, abdominal pain) limit the clinical application of this drug. These side effects might be overcome by use of nanoparticles for tumor delivery of sorafenib [1]. Here, we reported the preparation, chemical-physical and technological-pharmaceutical characterization of Nanostructured Lipid Carriers (NLC) containing sorafenib. The lipid matrix was composed of tripalmitin (solid lipid at room temperature) and Captex 355 EP/NF (liquid lipid at room temperature). By in vitro experiments we demonstrated that these nanosystems have modified the sorafenib release, protecting it from degradation by plasma enzymes and by protein binding. By Scanning Electron Microscopy we evaluated their spherical shape, also confirming the nanometer size. Finally, we performed in vitro studies (i.e. MTS assays and clonogenic assays) on human hepatocellular carcinoma cells to evaluate the cytotoxicity of the free drug, empty and drug-loaded NLC. These studies demonstrated an improved therapeutic efficacy of sorafenib-loaded NLC compared to the free drug and suggest that lipid nanoparticles could have a great potential as sorafenib targeted delivery systems.

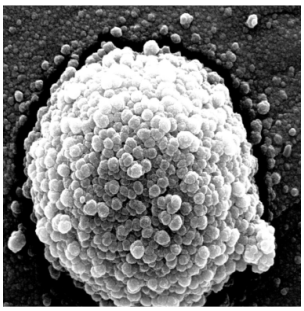

Figure 1. SEM analysis of sorafenib loaded NLC

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Analysis of the bacterial methanogenic community in a vegetable biomass and swine manure digester

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Keywords: **Biomass, Methanogens, Renewable**

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 production by biomass utilization can widely reduce carbon dioxide emissions and consequently can prevent global warming (1). Biomass can biologically be converted to biogas (CH_4, CO_2). Among different conversion processes for biomass, biological anaerobic digestion is one of the most economic way to produce biogas from various biomass substrates. Different consortia of microorganisms with different function in the anaerobic digestion process are needed. In addition to hydrolyzing, fermenting microorganisms and acetogenic bacteria, the activity and performance of the methanogenic bacteria is of paramount importance during methanogenesis. Methanogenic Archea are obligately anaerobic microorganisms that obtain their energy for growth from the conversion of a limited number of substrates to methane gas. The major substrates are H_2+CO_2, formate, acetate. The aim of our project***** is to isolate and characterize the methanogens species present in samples from a biodigester containing vegetable biomass and swine manure. Any enrichment cultures of methanogens, through the use of anaerobic techniques (2), 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 in a mineral salt medium. The culture did not use acetate as a growth substrate.

*****project financed with funds from the cross-border Cooperation Programme **ENPI - CBC** Italy-Tunisia 2007/2013

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Oxysterol mixture in hypercholesterolemia-relevant proportion causes oxidative stress-dependent eryptotic activity.

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Keywords: **oxysterols, eryptosis**

Oxysterol toxicity on erythrocytes (RBCs) has not been studied yet. Eryptosis (RBC programmed death) is characterized by membrane phosphatidylserine (PS) scrambling and cell shrinkage. Eryptotic effects of oxysterols, in a mixture compatible with that in plasma of hyper-cholesterolemic patients, or individually, were investigated on healthy human RBCs, either isolated or after *ex vivo* spiking of whole blood.
Methods: PS exposure, calcium entry, ROS production, amino-phospholipid translocase (APLT) activity were evaluated by cytofluorimetric assays, cell volume from forward scatter. Prostaglandin PGE2 was measured by ELISA; GSH-adducts and lipoperoxides by spectrophotometry. Involvement of protein kinase C and caspase was investigated by pre-treatment with inhibitors staurosporin and Z-DEVD-FMK, respectively.
Results: Oxysterols caused PS scrambling, cell shrinkage, and hemolysis to a lesser extent. Eryptosis was associated with PGE2 release, opening of PGE2-gated calcium channels, ROS production, GSH depletion, membrane lipid oxidation. Calcium removal prevented cell shrinkage, with small effect (-20%) on the PS

externalization, and did not affect ROS generation. Conversely, addition of antioxidants fully prevented Ca^{2+} influx and eryptosis. Either staurosporin or Z-DEVD-FMK blunted PS externalization. Oxysterols inhibited APLT and inward transport of PS. Only 7-ketocholesterol and cholestan-3β,5α,6β-triol were individually active. Significant eryptosis was observed *ex vivo*.
Conclusions: Oxidative stress triggers oxysterol-induced eryptosis, with coordinated and complementary processes not strictly dependent on calcium activity. Potential relevance for eryptotic activity of oxysterols *in vivo* is suggested.

Study and testing of medical devices for remote monitoring of patients with chronic conditions.

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Keywords: **Telemedicine, medical devices, health care**.

Remote healthcare systems have received increasing attention in the last decade, explaining why intelligent systems for e-health care are an emerging area of development(1). Telemonitoring is a tool based on the use of communication technologies to monitor simple clinical variables, in order to enable early detection of any alteration, providing an opportunity to prevent hospitalization(2). We used a physiological status monitor (PSM) embedded into a compression shirt to enable patients to measure and transmit vital metrics. We monitored 12 individuals and each individual was monitored for 8 hours. The components of the PSM were: 1) two sensors for the detection of ECG and the heart rate (HR), 2) an electric piezo-resistive sensor for respiratory rate (RR), 3) a triaxial accelerometer for posture of the individuals, 4) a control unit, integrated with the shirt, that recorded all signals in an internal memory and transferred the data to an external unit by Bluetooth. The subjects were enabled to introduce markers during the recording, whenever they felt discomfort. All the data were than transferred to a medical records repository for an easy consultation. The physician could access the repository, in a secure way, to analyze the record and evaluate any significant alteration of the ECG, HR and RR. It would be interesting to make a cloud network that allow patients to share their vital parameters in the net and medical personnel to be able to see the information of each individual, through the electronic health dossier, in real time.

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Codon 12 and codon 13 mutations in K-RAS differentially affect therapies response of colorectal carcinoma cells.

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Keywords: **Colorectal-cancer, K-RAS, Cetuximab**.

The colorectal cancer (CRC) is a heterogeneous disease, which develops as a result of numerous genetic and epigenetic alterations. A gene family frequently mutated in tumors is that of ras which consists of three main oncogenes (H-, K-and N-Ras) on different chromosomes and encode 21 KDa G-proteins. The RAS proteins are involved in a highly conserved signal transduction pathways that regulates proliferation, differentiation and apoptosis and their activating mutations have oncogenic effects. K-RAS mutations, usually point mutations in codons 12 or 13, are common in CRC. Several studies show that mutations in different codons or different mutations in the same codon of K-Ras may have different biological effects and lead to a different response

to therapies based on Cetuximab, an anti-EGFR monoclonal antibody. K-RAS mutations are in fact considered to be a resistance factor to this drug, but it has been reported that tumors with codon 13 K-RAS mutations might be less resistant to Cetuximab than tumors with codon 12 mutations. To gain more information on this point, we analyzed HT-29 clones stably transfected with cDNAs codifying K-RASG12V (clone K12) and K-RASG13D (clone K13) under the control of an inducible promoter. We observed that Cetuximab affects the cell cycle and proliferation of the K13 clone expressing K-RASG13D but not the K12 cells induced to express K-RASG12V. These results support the hypothesis that tumors with different K-RAS mutations could respond differently to therapies. Moreover preliminary data show that Cetuximab has no effect on the rate of cell proliferation of the parental HT29 cell line, in which the Ras genes are wild type but there is a mutation in BRAF, nor on its cell cycle. However, western blot analysis show a Cetuximab effect on the level of expression and phosphorylation (i.e., activation) of the tumor suppressor protein P53, but not on that of one of the P53 targets, the cell cycle regulatory protein P21.

Bacteria consortia and deterioration of archaeological waterlogged wood: identification by molecular and microscopy techniques

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Keywords: **Waterlogged wood, Biodeterioration, Molecular investigation**

In this study molecular tools are applied to reveal and identify bacterial colonization in waterlogged wood to assessing the changes induced in anatomical structure, previously observed by Optical and Scanning Electron Microscopy (1). The results obtained by observation of wooden thin sections (OM), shown the presence of black and dark-brown areas and mineral concretions. The SEM analysis revealed a specific cell walls alteration, attributable to bacterial activity, other than abundant pyrite framboids (FeS_2). The presence of sulfur compounds in archaeological waterlogged wood can indicate both long-term burial in anoxic environment and colonization by sulfate-reducing bacteria. Molecular methods allow us extract microbial genomic DNA from wood samples and *in vitro* amplify (PCR) bacteria DNA target sequences (16S, ITS-rRNA) (2). Through sequences analysis of PCR products cellulosolytic and ligninolytic bacteria, such as *Pseudomonas*, *Cellulomonas*, *Xanthomonas* and *Bacillus* spp, have been revealed. Moreover the presence of *Marinobacter* sp. and *Desulforudis audaxviator*, respectively iron-oxidizing and sulfate-reducing bacteria, are identify. We hypothesize that this investigation approach, can be applied to a variety of wooden artifacts of archaeological findings for both characterization of microbial colonization in order to understanding the main degradation phenomena, indispensable for a correct conservation strategies.

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The miRNA profile associated with MBP-1 expression in breast cancer SKBR3 cells

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Keywords: **MBP-1, Breast cancer, miRNA-mRNA expression profiles**

Myc-promoter-Binding Protein-1 (MBP-1) is an alternative translated product of ENO1 mRNA which acts as a transcriptional repressor of c-MYC, ERBB2 and COX2 genes (1). Exogenous MBP-1 expression suppresses proliferation and induces apoptosis in several cancer cell lines. In infiltrating ductal carcinoma (IDC), the loss of MBP-1 nuclear expression significantly correlates with adverse outcome, suggesting that MBP-1 may play a role in breast cancer progression and making it a novel prognostic marker for breast cancer (2).
MicroRNAs (miRNAs) are a large class of small non-coding RNAs that post-transcriptionally regulates gene expression in different biological processes involved in oncogenesis (3). In prostate cancer cells it has been shown that MBP-1 upregulates miR-29b expression, which in turn inhibits the expression of anti-apoptotic and pro-metastatic proteins (4).
In a our previous study, we observed no correlation between MBP-1 expression and miR-29b level in MBP-1^{+ve} and MBP-1^{-ve} primary IDCs, as well as in SKBR3 breast cancer cells ectopically expressing the protein. To investigate the existence of a functional relationship between MBP-1 and miRNAs in breast cancer, a microarray analysis was conducted on SKBR3 cells relative to MBP-1 protein expression. The analysis of the miRNAs expression profile was also integrated with the analysis of differentially expressed mRNAs.
Our data indicate that MBP-1 plays a role in pathways frequently altered and associated with the onset and the progression of the malignant disease, by affecting, directly or indirectly, the expression of several miRNAs and mRNAs in a c-Myc-independent manner.

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Monitoring the quality and shelf life of crustaceans with commercial interest by the HSC70 protein levels

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Keywords: **food quality, freezing and thawing, crustacean freshness**

Within the DeCroMed Project, we used a multi-parametric approach to study the quality of the shrimp *Parapaeneus longirostris*, and the lobster *Nephrops norvegicus*, during post-thawing periods. Nowadays, there is a lack in the knowledge of the changes taking place during handling, processing and storage of crustacean species of commercial importance caught in the Mediterranean Sea. The aim of this study was to obtain detailed information, especially at the biochemical level, on the crustacean post mortem changes occurring after thawing procedures aimed at monitoring crustaceans freshness and shelf life over time. We measured the water-holding capacity of *P. longirostris* and *N. Norvegicus* muscles and monitored changes in pH, protein content and composition of muscle exudates obtained from each specimen by centrifugation. In addition, we evaluated the levels of a few candidate marker proteins chosen from a list of biological markers validated to predict the quality of food products. We found that HSC70 protein levels are negatively related to: i) the increase of the water-holding capacity, ii) the formation of protein aggregates and protein degradation, iii) the pH of muscle exudates, and iv) the flesh softening of the crustacean muscle. In

conclusion, we propose the HSC70 protein as a biomarker of crustacean freshness. Further studies are underway to identify additional biomarkers to evaluate the thawed crustacean quality.

Work fully supported by PO-FESR 2007-2013 – SICILIA- DeCroMed "Pescato Mediterraneo: certificazione di qualità e valorizzazione dei crostacei decongelati"; Linea di Intervento 4.1.1.1, GURS 28/10/2011,year 65, N° 45; CUP: G93F12000190004. Authors acknowledge the technical assistance of Mr. Mauro Biondo.

Apoptosis rate of cumulus cells can be considered as an indicator for the selection of embryos to improve ongoing pregnancy and implantation rates.

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Key words: **Reproductive medicine, embryo quality, molecular evaluation**.

Cumulus cells apoptosis rate an adjunct to morphology evaluation for the embryo selection on day 3 could be considered a new tool, compared with embryo selection by morphology alone, to select the embryos with higher implantation potential to increase the clinical outcomes after ICSI.
Several studies have demonstrated a lower cumulus cell apoptotic rates in women who achieved pregnancy compared with women who did not become pregnant after ICSI.
A prospective randomized observational study on 76 ICSI patients, undergoing *in vitro* fertilization, was performed before Ovum Pick-Up. Patients were randomized into either the control group (embryo selection by morphology only, **A group**: 48 patients) or the treatment group (morphology plus cumulus cell apoptosis evaluation, **B group**: 28 patients). On Day 3, embryo transfer of a maximum of 3 embryo of grade A was performed.
Patient demographics and baseline characteristics were distributed equally over the two groups. No statistical differences were found between the group A vs group B in terms of FSH units for ovarian stimulation (1833 ± 754 vs 1927 ± 826), E_2 at hCG administration (1872 ± 788 vs 1787 ± 796), the numbers of oocytes collected (6.4 ± 2.1 Vs 6.7 ±3.7), the number of transferred embryos (A group: 126; B group: 69), the grade A transferred embryos (126 vs 69). No differences was found in the cumulative DNA fragmentation rate in the cumulus cells (16.39 ±12.9 vs 15.7 ± 11.3). Significative differences were found in ongoing pregnancy rate (33.3 vs 57.1) and implantation rate (12.6 vs 23.1).
Embryos selection according to cumulus cells apoptosis rate could help to identify competent embryos with higher implantation potential, suggesting a new diagnostic tool in IVF laboratories to increase the clinical outcomes reducing the number of embryos to transfer.

Entrapment of Tyrphostin AG 14-78 into Lipid Nanoparticles Improves its Antitumor Activity against Human Hepatocarcinoma Cells

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Keywords: **Nanostructured Lipid Carriers, Tyrphostin AG 14-78, drug release, hepatocellular carcinoma**

In hepatocellular carcinoma (HCC), different signaling pathways are de-regulated, and among them, the expression of the epidermal growth factor receptor (EGFR). EGFR is expressed at high levels in a variety of solid tumors. In HCC, the overexpression of this receptor has been associated with late-stage disease, increased cell proliferation, and degree of tumor differentiation. In addition, activation of EGFR pathway is a prognostic predictor of survival in patients with HCC. Therefore, EGFR represents a good potential molecular target for biologic therapy of HCC. Tyrphostins are protein tyrosine kinase inhibitors. Among them, the tyrphostin AG-1478, 4-(3-chloroanilino)-6,7-dimethoxyquinazoline, a competitive inhibitor of the ATP binding site in the kinase domain of EGFR, inhibits proliferation and induces death of liver tumor cells through EGF receptor-dependent and independent mechanisms[1]. Previous studies also revealed that tyrphostin AG-1478 has no cytotoxic effects per se against normal hepatocytes, while it prevents proliferation and induces apoptosis in human HCC cells. Tyrphostin AG-1478 is a lipophilic low molecular weight inhibitor of EGFR, preferentially acting on liver tumor cells. In order to overcome its poor drug solubility and thus improving its anticancer activity, it was entrapped into nanostructured lipid carriers (NLC) by using safe ingredients for parenteral delivery. Nanostructured lipid carriers (NLC) containing tyrphostin AG-1478 were prepared by using the nanoprecipitation method and different matrix compositions. The best system in terms of mean size, PDI, zeta potential, drug loading and release profile was chosen to evaluate the anti-proliferative effect of drug-loaded NLC versus free drug on human hepatocellular carcinoma HA22T/VGH cells. Thanks to the entrapment into NLC systems, tyrphostin AG-1478 shows an enhanced *in vitro* anti-tumor activity compared to free drug. These finding raises hope of future drug delivery strategy of tyrphostin AG-1478 -loaded NLC targeted to the liver for the HCC treatment.

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Heterogeneity of skeletal muscle-derived extracellular nanovesicles and role of protein lipidation

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Keywords: **Exosomes, Skeletal muscle, nanovesicle biogenesis, Alix, extracellular vesicles**

Several cell types secrete exosomes, which contain cell-specific proteins, lipids, and genetic material. Recently, we and others have shown that skeletal muscle (SkM) cell can release Alix-positive exosomes[1], revealing the importance of exosomes in skeletal muscle biology. Since skeletal muscle is now considered a secretory organ, discoveries on SkM-derived exosomes in health, disease, and regeneration would provide an important link between genetic and epigenetic factors. Furthermore, coupled to nanotechnology, engineered exosomes might be useful to allow the tissue regeneration, and to recover from muscle atrophy and/or injury.

Here, we investigated how muscle cells generate these vesicles and what their regulators are. The skeletal muscle cell line C2C12 were treated or not with specific inhibitors of protein lipidation, and then the SkM-derived exosomes were isolated at different stage of SkM differentiation, using differential ultracentrifugation. To characterize exosomes, we applied biophysical techniques, e.g. dynamic light scattering (DLS), coupled to immunoblot assays. To date, exosomes have mainly been studied with a biological approach; here, SkM-derived exosomes were analyzed by biophysical techniques under quasi-physiological conditions. The use of static and dynamic light scattering allow to assess the diffusional and structural properties of exosomes in solution, giving a measure of their size, their shape and size distribution. These informations and analyses of exosomes specific biomarkers/regulators, after the inhibition of protein lipidation, qualify these techniques as methods to analyze the size and integrity of exosomes in dispersion, in different experimental settings.

The research has been funded by the Italian Ministry for Education, University and Research in the framework of the Flagship Project NanoMAX.

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Supercritical carbon dioxide induces sterilization of PLLA scaffolds contaminated by *E. coli*.

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Keywords**: supercritical carbon dioxide, PLLA, *Escherichia coli*.**

The common sterilization techniques are based on physical processes that involve, for example, the use of autoclaves or systems to radiation such as *γ*-rays that can cause a structural change of the polymer treated. Therefore, the use of supercritical carbon dioxide ($scCO_2$) is an excellent alternative, as it does not induce any variation of biomaterials treated (1). It's a good candidate because is readily available at low cost, non-toxic and non-flammable, it has an easily accessible critical point (7.38 MPa and 304.2 K) and excellent transport properties and wettability (2).

We report the development of a supercritical CO_2 based process capable of sterilization of PLLA [poly(L-lactic acid)] scaffolds that can be used for tissue engineering applications. The PLLA scaffolds were contaminated by the gram negative bacterium *Escherichia coli* or environmental microorganisms: the amount of bacteria in each scaffold was determinated by colony-forming unit (CFU). Than they were subjected to different (for temperature or pressure values) supercritical CO_2 processes. A good sterilization was obtain with a pressure of 150 bar for only 15 minutes of treatment at 37°C.

The process does not alter crystallinity and melting temperatures of the scaffolds, as demonstrated by DSC analysis (differential scanning calorimetry) of the scaffolds treated and not. Therefore, the treatment does not significantly alter the properties of the sample.

The CO_2 treatment does not intact the biocompatibility of the scaffolds as demonstrated by MTS assay (viability assay) of SK-HEP-1 tumour cells growth on the surface and the internal pores of the scaffolds.

These results suggest that the $scCO_2$ can be used as a perfect method of sterilization for PLLA scaffolds for their possible use for tissue engineering applications.

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cis-Regulation and chromatin dynamics of the *hbox12* gene during the embryogenesis of *Paracentrotus lividus*.

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Keyword: **Dorsal-Ventral axis; Gene Regulatory Network; chromatin dynamics; sea urchin embryo**.

The GRN specifying the dorsal-ventral (D-V) axis of the sea urchin embryo is currently under investigation. An early input for D-V polarity is given by a redox gradient probably generated by an asymmetrical distribution of maternal mitochondria (1). Only on the future ventral side, the oxidizing environment induces the expression of the *nodal* gene, an essential regulator of D-V polarization (2). By contrast, on the future dorsal side, a reducing environment activates the hypoxia inducible factor (HIF-1α) (3). The *hbox12* transcription repressor is an early marker of the dorsal side of the embryo, in which it negatively regulates the expression of *nodal* (4, 5). Interestingly, by *in silico* analysis we identified an evolutionarily conserved HIF-1α binding element (HRE). Gene transfer assays also suggested that HIF1α stimulates *hbox12* expression. To map the physical interaction of HIF1α with HRE, a region of the *hbox12* promoter containing the HRE was cloned in a *Gaussia princeps* luciferase reporter system and the resulting construct was trasfected in HEK293 cells. Moreover, we cultured *P. lividus* embryos with two different HDAC inhibitors, VPA and TSA, and observed perturbation of the spatial-temporal expression profile of *hbox12*. Finally, by chromatin immunoprecipitation assays we determined an increase of the acetylation of the lysine 9 of the histone H3 and the failure of the HDAC-1 enzyme on the *hbox12* chromatin.

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Toll-Like 3 Receptor In The Immune Response of *Paracentrotus lividus Sea Urchin*

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Keywords: **Coelomocytes, Quantitative PCR**

Sea urchin innate immune response involves important proteins such as membrane receptors which trigger different intracellular signaling pathways. The discovery of the Toll-like receptors (TLRs) in immune cells of invertebrates such as the sea urchin, has renewed the interest in innate immunity and its potentiality in invertebrate models. The sea urchin coelomocytes are considered a good model to study the immune response (1, 2, 3, 4). In the sea urchin *Strongylocentrotus purpuratus*, these receptors are encoded by a large multigenic family which comprises 253 genes (5). Here we report the isolation, the protein analysis and the phylogeny of a partial cDNA encoding for Pl-tlr3 belonging to the family of Toll-like receptor from the coelomocytes of sea urchin *Paracentrotus lividus*, and its expression in response to LPS and poly-IC treatments. The analysis of the protein has allowed to understand that this receptor, localized into the endosome, is small in size and contains a small number of Leucine Rich Repeats. It is not involved in the immune response bacterial, given that its transcription, analyzed by QPCR, is not affected by the injection of LPS. On the contrary, poly-IC treatment, a chemical compound that mimes dsRNA, causes Pl-tlr3 overexpression. The phylogeny of the partial protein Pl-tlr3, compared with Tlr3 proteins from different classes of animals, give a vision of the evolutionary path that, most likely, this receptor has made in the course of millions of years, starting from simple organisms up to man. The study of the functions of Tlr3

genes in invertebrate may provide new perspectives on the knowledge of the TLR family of receptors in the immune response.

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Apoptosis is related to autophagy in sea urchin embryos exposed to cadmium

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Keywords: **apoptosis, autophagy, cadmium stress**

The sea urchin embryo is a suitable model system that offers an excellent opportunity to investigate different defense strategies activated in stress conditions. We previously showed that cadmium treatment provokes the accumulation of metal in dose-time dependent manner in embryonic cells and the activation of defense systems, such as the synthesis of HSPs and/or the initiation of apoptosis.
Analyzing autophagy, by neutral red, acridine orange and LC3-detection, we demonstrated that Cd-exposed embryos adopt this process as an additional stratagem to safeguard the developmental program. We observed that embryos treated with subletal Cd concentration activate massive autophagic response after 18h of treatment. In addition, autophagy decreases between 21h and 24h, in the opposite of apoptotic process (1-2). In order to investigate a possible temporal relationship between autophagy and apoptosis , we tested apoptosis by immunodetection in situ of cleaved caspase-3 and TUNEL assays we showed that embryos activate a massive apoptosis after 24h of Cd-treatment. In addition, a functional relationship between autophagy and apoptosis was estimated evaluating apoptosis in Cd-exposed embryos with inhibited autophagy, by treatment with 3-methyladenine (3-MA). We found that the inhibition of autophagy produced a reduction of apoptotic signals, suggesting that the two phenomena are functionally related. Considering the catabolic role, an energetic hypothesis to explain this relationship was evaluated; in this case autophagy could contribute to apoptotic process providing ATP, necessary for execution of the apoptotic program. In effect, using Methylpyruvate (MP), a substrate for ATP production, in embryos with inhibited autophagy, apoptosis was substantially restored. In this context, autophagy could play a crucial role in stress response of this suitable model system (3).

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Genetics of *Anadenanthera colubrina* var. *cebil* (Fabaceae) tree from Salta (Northwestern Argentina)

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Anadenanthera colubrina var. *cebil* is an important tree species for its cultural, economic, and medicinal uses in South America. The wood contains tannins, is hard and resistant to termites and is used in construction and furniture, as poles and for firewood. In popular medicine, it is used to treat respiratory injury and inflammations. It is considered a sacred tree by local cultures. Its seeds have been used for over 3.000 years by shamans in rituals and popular medicine (1). This species is categorized as "least concern" with low risk of extinction (IUCN, 2012) (2). In order to characterize *A. colubrina* populations, we collected fruits from four different sites (San Bernardo, El Cebilar, Metán and El Gallinato) within the species distribution area in Salta Province, Northwestern Argentina. A total of 75 fruits and seeds per site were collected and described using morphological (fruits size and weight; seed weight and number per fruit) and genetic descriptors (ITS-ribosomic DNA isolation, sequencing and phylogenetic analysis). Our previous results, obtained from a few number of individuals, showed that the San Bernardo population and Metan population are more similar each other as well as El Gallinato and El Cebilar populations, both at morphological and genetic levels (3).
Currently, a greater number of individuals were analyzed at genetic level: 40 samples were collected from the 4 studied zones. Preliminary outcomes, obtained through the partial phylogenetic analysis, comfirmed the similarity, indicating that the genetic characteristics of *A.colubrina* populations follow the geographic distribution of the plant and therefore the geographic variations linked to climate.

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Intra-individual Gene Expression Profiling of Peripheral Blood CD34+ Hematopoietic Stem Cells Mobilized by Two Different Protocols

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Keywords: **Hematopoietic stem cells, Mobilization, Gene Expression**.

Hematopoietic stem cells (HSC) are capable of self-renewal and to differentiate into all mature blood cells, and therefore widely used in bone marrow transplantation (BMT). Moreover, CD34+/HSC -targeted gene transfer is an attractive approach for the treatment of some disorders caused by single gene defects. The number, together with the capacity of homing and engraftment, of HSCs available for transplantation have been reported to be critical for the success of both BMT and gene therapy and, in the latter case, especially for diseases in which the HSC genetically corrected not have a selective advantage. Mobilized peripheral blood progenitor cells represents a desired source of HSC, due to higher yields of CD34+ and the atraumatic collection procedure, as compared to conventional BM harvest. Granulocyte-colony-stimulating-factor (G-CSF), and more recently, AMD3100 are the two agents used for mobilization, alone or in combination. They act via two different mechanisms and therefore they could release stem cells with different intrinsic features. In a clinical trial of gene therapy we are conducting in patients with β-thalassemia we mobilized the same patient with two different protocols: G-CSF (G) *vs* G-CSF+AMD3100 (G+A). We were interested in the intra-individual comparison of the gene expression profile of purified CD34+/G+A *vs* CD34+/G cells. Differentially expressed genes were analyzed by using Agilent platform, and we found a similar gene expression profiling between the two samples, although some differences exist that can be ascribed to the different mobilization protocols. Our microarray data have provided evidence that the combination of G+A agents mobilizes a subset of CD34+/HSC with favorable characteristics for transplantation; in fact, the CD34+/G+A show significantly higher levels of expression of several genes that are involved in promoting cell motility, homing and engraftment, and resistance to stress, compared to CD34+/G cells.

Statistical Validation of a Comprehensive Gene/miRNA Expression Profile Dataset for miRNA:mRNA Interaction Analysis

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Keywords: **miRNA regulation, RISC immunoprecipitation, interaction network**

MicroRNAs (miRNAs) are small non-coding RNA molecules mediating the translational repression and degradation of target mRNAs in the cell. Mature miRNAs are used as a template by the RNA-induced silencing complex (RISC) to recognize the complementary mRNAs to be regulated. Several prediction tools are available to suggest the miRNA targets, however, only a small part of them has been validated by experimental approaches. In addition, none of these tools does take into account the network structure of miRNA:mRNA interactions, which we believe is crucial to efficiently predict the miRNA regulation effects in a specific cellular context.
We aim to model the miRNA:mRNA interaction network, by including all the miRNAs and mRNAs endogenously expressed in any cellular condition. We started by using as test bed the breast cancer MCF-7 cells. In order to build the miRNA:mRNA interaction model, we collected several miRNA and mRNA expression profiles, by using the Agilent microarray platforms . We analyzed samples derived from the immunoprecipitation (IP) of two RISC proteins, AGO2 and GW182. Specifically, we considered the input, the IP and the flow through samples. We also collected and analyzed miRNAs and mRNAs from polysomal/non polysomal fractions separated through sucrose gradient, as completion of a dataset useful to investigate on miRNA function. The expression level of the top expressed miRNAs has been validated by real time PCR.
Due to the peculiarities of our dataset, we used non-standard bioinformatics techniques to preprocess and analyze the obtained expression profiles. As result, we validated the sample extraction techniques (both RISC proteins IP and polysomes isolation), by obtaining expression profile clustering and regression results consistent with the experimental design. Our dataset can then be used to further investigate on miRNA:mRNA interactions, and here we also show our preliminary results in this direction.

Developmental Effects of Kenpaullone, a member of the family of benzazepinones, on Sea Urchin Embryos.

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Keywords: **sea urchin embryo, drug assay**

In a search for compounds able to inhibit epithelial mesenchymal transition in sea urchin embryos, we tested the *in vivo* activity of Kenpaullone (9-bromopaullone), described as a potent inhibitor of glycogen synthase kinase 3β and ATP-competitive inhibitor of Cdk1/cyclinB (1). GSK3α and β isoforms are phylogenetically closely related to the cyclin dependent protein kinases Cdk1 and Cdk2 (2).
In the field of cancer, protein kinases have become the most important class of drug target for the pharmaceutical industry and it is increasingly evident that Cdks, cyclins and their inhibitors play key roles in transcription, epigenetic regulation and other important cellular processes in mammals (3). Nevertheless, careful analysis and comparisons of the effects of inhibitors on growing cells (4) and animal models should be exploited. Among other molecules, Kenpaullone (Kp), has drawn our attention. In preliminary screenings, treatment of *Paracentrotus lividus* embryos at different drug concentrations from fertilization up to 16-20 h, led to the development of empty living blastulae, suggesting that Kp does not inhibit cleavage. Pre-hatching treated embryos were drug sensitive showing developmental defects after few hours, while hatched embryos were more robust. In order to define time sensitivity windows linked to specific developmental effects, Kp was added at early developmental stages and different interval times, then removed. Embryos were allowed to develop for morphological analysis and immunological staining. Results indicate that even very short treatments at early cleavage stages inhibit primary mesenchyme cells ingression and/or specification.

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Biotechnology for microbial monitoring of indoor cultural heritage environments

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Keywords: **Microbial monitoring, Preventive conservation, Human health**.

An integrated approach for the characterization of bioaerosol was employed in different sites (that include hypogeal and semi-confined areas), characterized by great cultural/artistic interest besides peculiar architectural structures, thermo- hygrometric and lighting parameters. These typologies of indoor environments preserve several artworks like mural paintings, stone-works, paper or parchments that are susceptible of microbial colonization. The presence of fungal spores and low air change can induce both potentially effects to human health (users/operators) or biodegradation of historical-artistic manufacts. We perform bioaerosol sampling by a portable sampler (Sartorius MD8), equipped with gelatin filters and non-invasive sampling (Nylon membrane or sterile swab) is carried out on works of art surface. Microbial consortia is revealed and characterized by Optical, Scanning Electron and Confocal Laser Scanning Microscopy (OM, SEM, CLSM), *in vitro* culture and molecular analysis (PCR, sequencing, sequence analysis). The inter-disciplinary approach applied in this study, represents a valuable contribution for the proper planning of both direct and/or indirect biological growth control and for the conservative restoration procedure (1, 2).

This work was supported by Research Project It@cha - Italian Technologies for Advanced application in Cultural Heritage Assets, grant PON 01_00625 "Ricerca e Competitività" 2007-2013.

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Seabream (*Sparus aurata*) hierarchies of social behaviour affects stress responses and immunity

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Keywords: **social stress, cortisol, phagocytosis**.

Fish are affected by environmental conditions that can cause stress, which leads to changes in the innate immune system and plasma parameters that increase susceptibility to disease. We examined behaviour under social stress in gilthead seabream (*Sparus aurata*). Social hierarchies ("dominant"; subordinate: "alfa" and "beta") were characterised by behavioural changes, such as "aggressiveness" and "feeding order", and were established after an hour of exposure to social stress. To characterise physiological stress, we measured the plasma levels of cortisol, glucose, and osmolarity, and we observed that the levels of these stress markers were higher in subordinate individuals, "beta" and "alfa", than in the "dominant" individuals. Four experimental models were used: in the first, the paired fish were placed simultaneously and

observation had a duration of six months; in the second, three fish were entered at the same time observation had a duration of 24 hours; in the third three fish were placed in a sequential manner and were observed for 15 days; in the fourth model two fish were placed simultaneously in a separate tank in two equal areas by a corrugated and transparent separator after four days was taken off the separator. In these experimental models, we have observed that social stress affects the levels of cortisol in the plasma and the activity of phagocytosis of peritoneal exudate cells demonstrating that social stress appeared to affect the cellular innate immune response of the subordinate individuals. In particular, the social stress more substantially affected the beta specimens. Finally, discriminant analysis clearly separated the subordinate fish groups from the dominant and control groups and a significant separation between the random and sequential models was observed. Moreover, modulation of phagocytic and respiratory burst activities revealed that social stress appeared to affect, in a time dependent manner, the cellular innate immune response of the subordinate specimens.

Identification of a prognostic gene signature associated with MBP-1 expression in ErbB2-negative breast carcinomas

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Keywords: **MBP-1, Breast cancer, mRNA expression profiles**

The ENO1 transcript, which encodes the glycolitic enzyme alpha-enolase, can be translated into a shorter nuclear protein called Myc-promoter Binding Protein-1 (MBP-1) by using an alternative translation start site. MBP-1 acts as a negative regulator of c-Myc, ErbB2 and Cox2 genes (1). Several evidences indicate that MBP-1 acts as a tumor suppressor in breast carcinoma and prostate cancer and its expression results in a reduced invasive ability (2). In our previous studies, we showed that MBP-1 is expressed and easily detectable in normal breast epithelial cells, but a loss of expression occurs in most primary invasive ductal carcinomas (IDC) of the breast. Furthermore, in these tumors MBP-1 expression inversely correlated with the expression levels of the ErbB2 and Ki67 proteins (3).

In order to better understand the role of MBP-1 in breast cancer and to correlate its expression to a gene signature with prognostic value, we evaluated the expression of approximately 21,000 genes in primary breast carcinoma by using the Agilent microarray technology. A comparison of the gene expression profiles obtained from MBP-1^{+ve} and MBP-1^{-ve} ErbB2-negative IDCs led to the identification of differentially expressed (DE) genes that may underlie the different clinical behaviors of these two subtypes of breast carcinoma.

Owing to the prognostic influence of nuclear MBP-1 expression in a subgroup of tumors from patients with node-negative and ErbB2-negative carcinomas, the combination of immunohistochemical analyses of MBP-1 and proteins encoded by genes we found DE by expression profiling, may prove to be a clinically valuable prognostic variable for breast cancer patients.

* partially supported by a grant from the Italian Ministry of Economy and Finance to the CNR for the Project FaReBio di Qualità, and from FIRB-MERIT n°RBNE08PA9T

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Biological effect of an Hybrid Anticancer Agent Based on Kinase and Histone Deacetylase Inhibitors on Breast Cancer Cells

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Keywords: HDACi, VEGFRi, breast cancer, MDA-MB231

Histone deacetylases (HDACs) are overexpressed in various types of primary human cancer and have become attractive targets for cancer therapy. This study examined the effects of an HDACi in combination with a vascular endothelial growth factor receptor inhibitor (VEGFRi) on MDA-MB 231 cells in the form of both a cocktail of separate inhibitors and a chemically-synthesized hybrid [1]. Cytotoxicity was tested by the MTT assay and flow cytometric analyses were also performed to gain insight into the mode of cell death. The results from the MTT assay showed that the drug cocktail was more cytotoxic than the hybrid drug with IC_{50} values of 10μM and 29μM for the cocktail and hybrid respectively. In particular, the results from the flow cytometric analyses of cocktail-treated cells indicated that i) almost half of the cell population underwent apoptosis, ii) there was a decrease in the amount of autophagy-related acidic vesicular organelles, and, iii) a collapse in mitochondrial membrane potential. The hybrid drug, on the other hand, was not equally effective and was not shown to induce apoptosis. Additional biological assays showed that exposure of MDA-MB231 cells to the drug cocktail also resulted in cell cycle perturbation and in reactive oxygen species production. In conclusion, our evidence shows that the drug cocktail is more effective on triple-negative MDA-MB231 breast cancer cells than the hybrid drug. Further analysis is required to understand the molecular basis of the differences of the mechanisms of action of the compounds and to optimise the structure of the hybrid drug.

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Inorganic phosphate is a trigger factor for *Microbispora sp.* ATCC-PTA-5024 growth and NAI-107 production.

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Key words: **Ribosomal Post-translationally modified Peptides (RiPPs), Phosphate, Polyphosphate**.

Abstract: NAI-107, produced by the actinomycete *Microbispora sp.* ATCC-PTA-5024, is a promising lantibiotic active against Gram-positive bacteria and currently in late preclinical-phase [1]. Lantibiotics (lanthionine-containing antibiotics) are ribosomally synthesized and post-translationally modified peptides (RiPPs) produced by Gram-positive bacteria belonging to the Firmicutes and Actinobacteria phyla. The biosynthesis of biologically active compounds is developmentally controlled and it depends upon a variety of environmental stimuli and conditions. Inorganic phosphate (Pi) usually negatively regulates biologically-active molecule production in Actinomycetes [2], while it has been reported to have a positive control on lantibiotic production in Firmicutes strains [3]. So far, no information is available concerning the Pi effect on lantibiotic biosynthesis in Actinomycetes.
Starting from the mineral composition of Maltose-Glutamate (MG) medium, already used for other actinomycetes [4], we developed a suitable defined medium named NG-20. Pi-limiting conditions were established and confirmed by quantitative analysis of polyphosphate accumulation and of expression of selected Pho regulon genes, involved in the Pi-limitation stress response. Then, the effect of Pi on *Microbispora* growth and NAI-107 biosynthesis was investigated in NG20 containing increasing Pi amounts. Altogether, our analyses revealed that phosphate is necessary for growth and positively influences both

growth and NAI-107 production up to a concentration of 5 mM. Higher Pi concentrations were not found to further stimulate *Microbispora* growth and NAI-107 production. These results, on one hand, enlarge the knowledge on *Microbispora* physiology, and, on the other one, could be helpful to develop a robust and economically feasible production process of NAI-107 as a drug for human use.

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Inulin Coated Gold Nanoparticles As a Potential Tool For Cancer Therapy

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Keywords: **gold nanoparticles, drug delivery system, Photothermal therapy**

Gold nanoparticles (GNPs) have unique optical and chemical-physical properties that make them appealing for biomedical applications. The noble metal core is biologically inert, contributing to low toxicity and good biocompatibility [1]. Their large surface area and their high affinity for thiol groups allows their easy surface functionalization, so that therapeutic agents, targeting ligands and biocompatible coatings can be introduced [2]. GNPs also display the phenomenon of Surface Plasmon Resonance (SPR), resulting in large absorption band in the visible and near-Infra Red (NIR), whose wavelength depends on the GNPs dimensions and shape [3]. When laser-irradiated, GNPs convert the absorbed radiation into heat [4,5]. However GNPs cannot be administered in vivo without a proper coating, due to their low stability in aqueous media. In the present study the synthesis and characterization of a new inulin thiol derivative able to coat GNPs is reported, forming a colloidal gold based drug delivery system suitable as a potential tool for cancer therapy. For this aim, inulin was chemically modified in the side chain with primary amine groups (INU-EDA) [6] and some of these were used as a reactive moieties to introduce thiol groups by the pyridyldithiopropionate groups in inulin backbone (INU-EDA-PDP), necessary for chemical surface modification of GNPs. The free amine groups can be potentially exploited to introduce targeting ligands and to payload anticancer therapeutic agents. Exploiting the favorable GNPs properties, such as photothermal and optical properties, localized hyperthermia and simultaneous imaging of cancer cells can be achieved.

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Gene and Protein Signatures Associated to Treatment of MDA-MB231 Breast Cancer cells with JAHA , a novel Histone Deacetylases Inhibitor

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Keywords: **HDACi, Differential Display PCR, Real Time PCR, Proteomic analysis, gene and protein expression patterns**

Jay Amin hydroxamic acid (JAHA) [1] is a novel metal-based SAHA analogue synthesized in vitro, that shows significant cytotoxic activity [2] on MDA-MB231 breast cancer cells. To identify protein signatures associated to its cytotoxic activity, we utilized a proteomic approach to reveal protein expression changes after 18, 24 and 48 h of exposure. The protein identification was performed by mass spectrometry, and a total of eleven differentially-expressed proteins were visualized. Subsequently, Differential Display (DD) gene expression analysis was used to identify gene signatures in MDA-MB231 human breast cancer cell line after exposure to JAHA. We found two genes, Rad50 (DNA repair protein) and NTRK2 (neurotrophic tyrosine kinase, receptor, type 2), upregulated in treated cell preparations, and five genes that encode for Protein kinase C epsilon type, Protein kinase C iota type, Ergic (ER-Golgi intermediated compartiment 2KDa protein), MED25 (Mediator of RNA Polymerase II transcription subunit25) and Brefeldine A-inhibited guanine nucleotide-exchange protein 3 that were significantly down-regulated after treatment with JAHA.The result obtained by DD-PCR will be confirmed by Real Time PCR analysis. Further study will be required to compare the reported signature pattern with that obtained after exposure of MDA-MB231 cells with the parental molecule SAHA, and to understand the biological implications of the expression changes found.

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The effect of the HDACi JAHA on DNA Methylation of breast cancer cells by down-regulating DNMT1 through ERK signaling

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Keywords: **HDACi, DNA Methylation, DNMT1 level, ERK signaling, MDA-MB231 breast cancer cells**

Methylation of CpG repeats in the upstream/promoter regions of genes is an established mechanism of gene silencing in many cell types. DNA methylation results in the recruitment of histone deacetylases (HDACs) to promoter regions, thereby repressing expression of genes. General inhibitors of class I and II HDACs (HDACi), suppress the growth of cancer cells in vitro and in vivo. In this study, we investigated the effect of JAHA [1], a novel HDACi, on the intracellular signaling pathways of MDA-MB231 breast cancer cells. Concerning the MEK pathway JAHA repressed MAP kinase (ERK) activation after 18 h until 30 h of the treatment, and also down-regulated DNA (cytosine-5-)-methyltransferase 1 (DNMT1), a downstream ERK target, already at 18h with an increase up to 48 h. To check the occurrence of changes in the extent of global DNA methylation, genomic DNA was submitted to MeSAP (Methylation Sensitive Restriction Arbitrarily-Primed) PCR [2] using Afa and then HpaII enzymes followed by PCR amplification with an arbitrary primer binding preferentially to guanine and cytysine (GC)-rich regions of DNA, including CpG islands. Preliminary indications suggest the ability of JAHA to induce hypomethylation patterns in tumoral breast cancer cells after 30 h of the treatment. Collectively, these data demonstrate that the HDACi JAHA, by inhibiting ERK activity, regulates DNMT1 expression and ultimately DNA methylation.

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A microRNA and mRNA signature of neuroblastoma LAN-5 cells expressing MBP-1

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Keywords: **ENO1/MBP1; Neuroblastoma; Microarray**.

An alternative translated product of the ENO1 gene, known as MBP-1 (c-Myc-promoter Binding Protein-1), acts as a negative regulator of the c-MYC oncogene, ERBB2 and COX-2 genes (1). The ENO1 gene is located in chromosomal region 1p36.2, within the common region of deletion detected in Neuroblastoma (NB) often associated with the amplification of MYCN gene (2).
We have previously shown that MBP-1 interacts with MYCN gene promoter and negatively regulates its expression acting as an oncosuppressor protein in LAN-5 cells. Furthermore, in NB cells MBP-1 overexpression significantly reduces migration and proliferation and ultimately induces cell death.
To investigate the functional role of MBP-1 in NB, we performed global gene and miRNA expression analyses, using microarray technology, to identify genes and miRNAs that are differentially expressed (DE) in LAN-5 cells in the absence or in the presence of MBP-1 expression. The analysis of the gene expression profiles confirmed that MBP-1 expression results in MYCN downregulation and $p21^{cip}$, BAX and γ-enolase overexpression and allowed us to identify other MBP-1 putative targets.
The miRNA expression analysis resulted in the identification of several miRNAs with tumor-suppressor activity. In addition, a comparison of the pathway enrichment analyses obtained from MBP-1 DE genes and MBP-1 DE miRNA targets provided information on the biological functions of these genes and highlighted novel interesting regulative networks.
In general, the expression profiles analyses confirmed that MBP-1 plays an important role in cell growth, the activation of apoptosis and in the migration potential of NB LAN-5 cells, making it a good candidate as a therapeutic target for neuroblastoma.

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Stimuli and signaling pathways involved in the translocation of α-enolase to plasma membrane in non tumorigenic and cancer breast cells

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Keywords: **α-enolase, cell surface, signaling pathways.**

Alpha-enolase is a highly conserved glycolytic enzyme involved in multiple functions (1). Besides the mainly cytoplasmic localization, the protein has been detected on the cell surface of prokaryotic and eukaryotic cells, where it functions as a plasminogen receptor. Plasminogen bound to the cell membrane activates the enzyme system involved in systemic infection and cell spreading by degrading fibrin and extracellular matrix (2). Elevated expression of α-enolase has been observed in many tumor types and its surface expression has been reported in lung, pancreatic and breast cancer. Recently, involvement of surface α-enolase in invasion and metastasis has been demonstrated in lung cancer, although the molecular mechanisms of its translocation to the cell surface remain elusive (3).
In order to investigate how α-enolase moves to the plasma membrane and to identify the signaling pathways involved in this process, we stimulated mammary epithelial and breast cancer cells with receptor ligands and analyzed variations in the level of surface α-enolase.

In this study, we show that Epidermal Growth Factor (EGF) and pro-inflammatory endotoxin Lipopolysaccharide (LPS) treatments up-regulate the cell surface expression of α-enolase. In addition, both in non tumorigenic and cancer breast cells we observed that EGF- and LPS-induced cell motility is correlated with an increased level of α-enolase on the plasma membrane, consistent with the enolase function of plasminogen receptor. LPS-induced cell surface expression of α-enolase has been well documented in monocytes during the inflammatory response, here, we unravel for the first time the putative signaling pathways underlying α-enolase translocation to the cell membrane in mammary epithelial cells.

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Copy Number Variants and microRNAs in Autism Spectrum Disorders: a whole-genome analysis.

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Keywords: **Copy Number Variants, microRNA, Autism, Monte Carlo simulation**

In recent years, there has been an increased interest by the scientific community on Autism Spectrum Disorders (ASDs), neurodevelopmental disorders of childhood with an incidence of about 1/160 children [1]. Different studies have indicated a strong genetic basis for autism susceptibility, also supported by the presence of autistic features in several monogenic disorders (e.g.,Fragile X syndrome, Tuberous sclerosis). Since 2007 Copy Number Variants (CNVs) were recognized as important genetic factors in ASD [2].
Studies performed so far have highlighted the pathogenic role of CNVs in terms of dosage change for protein-coding genes and few works have suggested the potential involvement of miRNA on the pathogenesis of ASD [3]. Here, a novel computational procedure based on Monte Carlo randomization [4] was developed and applied to several published datasets to assess the potential pathogenic role of microRNA genes overlapping *de novo* Copy Number Variants (CNVs) in patients with autism spectrum disorders. Our results include the identification of 46 miRNA genes overrepresented in *de novo* CNVs from several chromosomes and we propose that at least 7 miRNAs from the latter group are likely to play a pathogenic role in autism. Moreover, the procedure used in this study can be effectively applied to CNV/miRNA data in other genomic disorders beyond ASD.

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Ni^{II}, and Zn^{II} Schiff Base Complexes: Telomeric G-quadruplex Stabilizers

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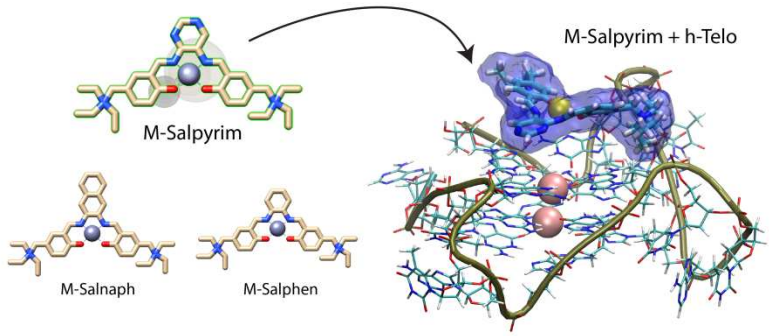
Keywords: **G-quadruplex, DNA, Metal complexes**

According to the World Health Statistics 2013, cancer is among the top three global leading risks for mortality, killing nearly eight million people a year worldwide. Recent years have seen an increased interest in non-canonical DNA structures, which can be considered excellent biological targets for selective anticancer drugs, due to their involvement in cellular carcinogenic pathways. In particular, footlights are turned on G-quadruplexes (G4s): G-rich sequences capable of forming four stranded structures organized in stacked guanine tetrads connected by looping DNA bases. G4 forming sequences are enriched at telomeres, where they inhibit the activity of the telomerase, but are also found in promoter regions of a number of genes (i.e. *c-Myc* and *c-Kit*) with functions in transcriptional regulation [1,2].
Schiff base complexes derived from *N*,*N*'-bridged tetradentate ligands, involving an N_2O_2 donor atoms, present very favourable features to act as G4 binders. We have reported on the B-DNA interaction ability of a series of Salphen-like metal complexes [3]. Properly modified ligands were synthesized to evaluate their G4 stabilization capability (see picture below).



Recently, Ni^{II} and Zn^{II} metal complexes of the ligand Salpyrim have been synthesized and characterized. Their affinity for wild-type *h-Telo* G-quadruplex DNA and for calf thymus DNA was investigated by UV absorption spectroscopy, circular dichroism and viscometry. The data collectively suggest that both complexes bind effectively to G-quadruplexes by direct end-stacking, stabilizing the oligonucleotide secondary structure. The two complexes are also typical B-DNA intercalators. Remarkably, their binding constants, K_b, with the G4s structures are about 10 fold higher than those with B-DNA, highlighting the selectivity. Experiments to evaluate the biological activity of the two complexes against MCF7 and HeLa cancer cell lines are currently ongoing.

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Cigarette smoke affects IL-17A, IL-17F and IL-17 Receptor expression in the lung tissue of COPD patients: ex vivo/in vitro *studies.*

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Keywords: **Cigarette smoke, IL-17, COPD**

Introduction: IL-17A and IL-17F target structural and inflammatory cells by IL-17R. Th-17 cytokines are involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). Cigarette smoke is a risk factor for COPD.

Aim: To evaluate the role of cigarette smoke on the expression of IL-17A, IL-17F and IL-17R in central and distal airways of COPD patients.

Methods: The epithelial and sub-epithelial immunoreactivity for IL-17A, IL-17F and IL-17R was assessed in surgical specimens from COPD patients (n=15), healthy smokers (HS) (n=10) and from healthy subjects (HC) (n=10) by immunohistochemistry. In vitro, human bronchial epithelial cell line 16HBE, human alveolar basal epithelial cell line A549 and peripheral blood mononuclear cells (PBMC) from normal donors were stimulated with cigarette smoke extract (CSE) (0, 2.5, 5, 10%) to evaluate the IL-17A, IL-17F and IL-17R expression by flow-cytometry.

Results: In central airways, immunoreactivity of IL-17A, IL-17F and IL-17R significantly increased in the epithelium of COPD and HS than in HC and IL-17A and IL-17F significantly increased in sub-epithelium of COPD than in HS and HC. In distal airways, immunoreactivity of IL-17A, IL-17F and IL-17R significantly increased in the epithelium and sub-epithelium of COPD than in HS and HC. However in distal airways, a trend toward higher levels was observed in HS than in HC. Finally, CSE significantly increased IL-17A, IL-17F and IL-17R expression in 16HBE, A549 and PBMC.

Conclusions: Increased expression *of IL-17A, IL-17F and IL-17R might be triggered by cigarette smoke, affecting lung epithelial and inflammatory cells in lung tissue of COPD patients.*

Ferulic acid inhibits constitutive and inducible IL-6 production in LPS-stimulated RAW 264.7 macrophages.

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Key words: **Antioxidants,Ferulic Acid, Raw 264.7**

Identification of new antioxidant and anti-inflammatory bioactive molecules is an important tool for selecting effective formulations for the treatment of inflammation. Mouse macrophage cell line RAW264.7 lipopolysaccharide (LPS)-activated is associated with a significant LPS-induced inflammation response. Activated macrophages produce reactive oxygen species (ROS), nitric oxide (NO) and inflammatory cytokines such as IL-6, TNF- α and IL- 10. In the present study, we showed that pre-treatment with ferulic acid (FA) reduces NO accumulation in the culture medium of LPS-induced macrophage cells. Moreover, real time experiments showed that FA has an inhibitory effect at the transcriptional level on the expression of some inflammatory cytokines, such as IL-6. Importantly, we found that FA reduced the translocation of nuclear transcription factor-κB (NF-kB) into the nuclei and inhibited IL-6 promoter activity in a luciferase assay. Previous data obtained from UV-Vis spectroscopy indicates that FA binds to DNA by intercalation (1,2). Ours data suggest that FA anti-inflammatory effects are mainly mediated through NF-kB pathway. Thereby,

considering the binding activity, FA could be a new DNA-targeted drug used to improve anti-inflammatory treatment.

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WIN modulates osteosarcoma MG63 cell migration by inhibiting MMPs activity and adjusting intra- and extra-cellular SPARC differential expression

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Key words: **osteosarcoma, cannabinoids, cell migration**

Invasion of cancer cells into surrounding tissue is an initial step in tumor metastasis. This event, which requires migration of cancer cells and attachment to extracellular matrix (ECM), is regulated by elements of the local microenvironment, including ECM architecture.
After having demonstrated the ability of the synthetic cannabinoid WIN55,512 to induce osteosarcoma MG63 cell death (1), we studied the effects of WIN on MG63 cell migration. Wound healing assay was performed to measure the ability of cells to migrate and fill the gap obtained by physical disruption of cell monolayer (2). We observed a significant delay in wound closure in 5 M WIN treated cells compared to untreated cells that almost completed the healing in 24 hours (20% *vs* 87% of wound closure). The addition of conditioned medium obtained by confluent control cells to WIN treated cultures largely reverted the delaying WIN action. To evaluate the influence of matrix metalloproteinases (MMPs) in the migratory ability, we analyzed MMP-2 and MMP-9 activities by zymography in WIN-treated culture medium. MMP-9 zymographic activity was strongly lower in WIN-treated culture medium in comparison with medium from control cells, suggesting that WIN inhibits MMP-9 activity.
Since it is known that cell/ECM interactions are mediated by SPARC (Secreted Protein Acidic and Rich in Cysteine) that also modulates MMPs activity (3-4), we evaluated intra- and extracellular levels of SPARC in our experimental conditions. RT-PCR and western blotting analysis showed in WIN-treated cells an increase both in mRNA and protein expression of intracellular SPARC, while a decrease in extracellular protein level was observed. Studies are in progress to study the possible involvement of SPARC in MMPs activation and MG63 cell migration.

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Non invasive Raman spectroscopic detection of skin carotenoids in β-thalassemia patients

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Keywords:**b-thalassemia, Raman spectroscopy, body antioxidant status**

Because of continuous blood transfusions, thalassemia patients are subjected to severe body redox unbalance and peroxidative tissue injury by the secondary iron overload. A novel non invasive optical method, based on the Raman resonance spectroscopy (RRS), can accurately measure the level of carotenoids in skin (1). Since the body's antioxidants constitute a highly integrated network, the level of

catotenoids in the skin reflects the whole antioxidant status (2). Then RRS can be used as a reliable biomarker of antioxidant defense without the inconvenience of blood samples. Fifty-seven β-thalassemia patients (31 major and 26 intermedia) were recruited for RRS measurement by a Pharmamex BioPhotonic® scanner and skin carotenoid levels were measured as Skin carotenoid score (SCS), in comparison to health subjects matched for sex and age. SCS in the patients was about an half of the values of normal subjects (36,66±11.81 vs 14.18 ±6.39; P<0.0001 Student's *t*-test) and showed a strong inverse correlation with plasma ferritin (Spearman's r= -.5648, P=0.0001), with liver stiffness correlated to fibrosis, measured by transient elastography (r= -.3599,P=0.0192) and with plasma transaminase AST (r= -.04475, P=0.0003) and ALT (r= -.2963,P=0.0215). More importantly SCS in β-thalassemia patients had a direct correlation with the intra-cardiac iron amounts measured by T2 Star magnetic resonance imaging (r = .3649, P=0.0243), whereas no relationship was found between SCS and hepatic iron accumulation. In conclusion our study shows that carotenoid measurement in the skin by RRS can be a reasonable non invasive method to monitor thalassemia patients for iron-related body oxidative stress.

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METFORMIN MODIFIES THE APP EXPRESSION IN CELL CULTURES AND THE KINETICS OF AMYLOID-BETA AGGREGATION

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keywords: Alzheimer's disease, type 2 diabetes mellitus, metformin

The high prevalence of both Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) in the elderly population suggests that concomitant pharmacotherapy could be desirable. AD is the leading cause of dementia in the ageing and is characterized by gradual loss of cognitive functions. Ageing is a primary risk factor for the development of AD, as well as for a variety of pathological conditions, such as T2DM, that can potentially obscure a diagnosis of AD or interfere with AD pharmacotherapy. Patho-histological hallmarks of AD include widespread neuronal degeneration, extracellular amyloid plaques and intracellular neurofibrillary tangles, mainly composed by amyloid beta-peptide (Aβ) and Tau protein, respectively. Clinical and experimental biomedical studies indicate that metformin, a therapeutic biguanide widely administered for T2DM therapy, rises the generation of Aβ, thus increasing the risk of AD insurgence. Elucidating this interaction between metformin administration and Aβ production, can explain the strict link between T2DM and AD. In the present work we demonstrate that metformin affects the cell viability and the metabolism of the Amyloid Precursor Protein (APP), the protein originating the Aβ fragment, in human neuroblastoma LAN5 cells. We found that the APP over-expression causes the formation of Aβ polydisperse aggregates, in a dose dependent manner. In order to understand whether metformin is also able to directly interact with Aβ, we performed in vitro extrinsic fluorescence and light scattering experiments by incubating the amyloid peptide with and without metformin. We found that metformin increases the lag phase and reduces the growth rate of Aβ kinetics thus indicating the formation of a drug-Aβ complex. However, the final fluorescence intensity shows that the objects formed in presence of metformin are richer in on-pathway β-structures if compared with controls. Furthermore, light scattering measurements exclude that the increased fluorescence intensity could be attributable to the occurrence of species with higher hydrodynamic radius.

Degradation Studies of Novel Polymeric Nanoparticles based on Amphiphilic Polylactic acid-Polyaspartamide Derivatives

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Keywords: α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA), biodegradable polymers, nanoparticles

The use of biodegradable nanoparticles as drug delivery vehicles for biomedical applications has increased in recent years due to their biocompatibility, biodegradability and minimal side effects [1].
Several natural and synthetic polymers have been used to realize these polymeric carriers, and among them, polyaminoacidic copolymers were chosen to realize drug delivery systems with adequate performance in terms of drug release and compatibility [2].
In this work, polymeric nanoparticles based on α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA) [3] and polylactid acid (PLA) were realised starting from two novel polylactide-polyaminoacid conjugates containing respectively polyethylenglicole chains (PEG) or galactosylated PEG chains. In particular, these copolymers were obtained by chemical reaction of PHEA with PLA and subsequent reaction with polyethylene glycol monoamine (PEG-NH_2) or with GAL-PEG-NH_2, obtaining PHEA-PLA-PEG and PHEA-PLA-PEG-GAL copolymers, respectively.
Galactosylated and/or pegylated nanoparticles based on PHEA-PLA-PEG-GAL and PHEA-PLA-PEG, with spherical shape and nanometric size, were successfully prepared by high pressure homogenization– solvent evaporation method [2].
Chemical degradation studies were carried out on these particles as a function of the incubation medium and the nanoparticle composition, using different techniques such as 1H-NMR, SEC, FT-IR and PCS and results either in qualitative or quantitative terms reported.

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The *Paracentrotus lividus* metallothionein gene family: structure and expression

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Keywords: **sea urchin development, metal response, gene expression**

Metallothioneins are metal binding proteins that play a pivotal role in metal homeostasis and detoxification. Since their initial discovery, they have been extensively studied in a variety of organisms ranging from microbes to plants and animals. Organisms often possess multiple genes encoding metallothionein homologs with distinct properties, such as varying affinities for different metals, and in many cases different functions. Despite the plethora of available studies, very little information is known about sea urchin *P. lividus* MT (1, 2).
We previously identified five Pl-MT embryonic cDNAs and we studied their induction after cadmium treatment (3). Now we studied their expression during embryo development by RT-qPCR. MT4 to MT6 are not expressed during normal development (very low levels of MT5 at pluteus stage), while MT7 mRNA level strikingly increases throughout embryonic development and MT8 rises until gastrula stage and decreases thereafter.

Preliminary *in situ* hybridization experiment results indicate that both MT7 and MT8 are expressed in the archenteron at pluteus stage, but MT7 is expressed also in the oral ectoderm.
We also isolated and sequenced the corresponding MT genes: all the Pl-MT genes have a similar structure (4 exons separated by 3 introns, the last intron into the 3'UTR) having introns in the same positions as S. purpuratus genes, but they are different from other deuterostomes.
MT promoters were analyzed *in silico*: putative metal response elements (MRE), antioxidant response elements (ARE) were identified, but their copy number and positions are different between constitutive (MT7-8) and induced (MT4-5-6) genes.

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Analysis of the mechanisms through which K-RASG12V and K-RASG13D regulate the proliferation and cell death in cells HT-29.

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Keywords: **RAS, mutations and carcinomas**.

The three major isoforms of RAS (H-, K- and N-RAS) differ only in the last 25 amino acids which are the site of different post-translational modifications that lead to localization in diverse plasma membrane microdomains and activation of alternative pathways of signal transduction. This might explain, at least in part, the different biological effects of the RAS isoforms in the cells. RAS mutations are a common event in several tumours and in colorectal carcinomas the genetic alteration is a point mutation in codons 12 or 13 of K-RAS. These mutations lead to a constitutively active protein by inactivating its GTPase activity. To shed more light on the molecular mechanisms responsible for the different effects of RAS mutations, we have used stable clones of HT-29 (a human colorectal adenocarcinoma cell line in which the endogenous RAS genes are wild type) transfected with cDNAs codifying: K-RASG12V (clone K12) and K-RASG13D (clone K13) under the control of a Mifepristone-inducible promoter [GeneSwitchTM System (Invitrogen)]. We found that the two mutated isoforms of K-RAS induce different effects on the growth rate and on the cell cycle and a similar increase in the $p21^{CIP1/WAF1}$ expression. The increase of $p21^{CIP1/WAF1}$ protein expression and the effects on the cell cycle are not reduced by treatment with MEK or/and PI3K inhibitors. K-RASG12V and K-RASG13D also induce differential effects on the pro-apoptotic (MST2-RASSF1A-LATS1) and the anti-apoptotic (MST2-RAF-1) pathways. Finally, the data show an increase in total cell death in both induced clones, but K-RASG12V induce a significant increase in apoptosis while K-RASG13D in necrosis; the cytotoxic effect observed in induced K13 cells decreases in starved conditions.

Cell-to-cell communications among brain cells by extracellular vesicles

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Keywords: **Microvesicles, brain cells, oligodendroglioma cells**.

Brain capillary endothelial cells (BCECs) form the blood-brain barrier (BBB) in response to interaction with other brain cells (astrocytes, pericytes and neurons). Neurons and astrocytes, in vitro, co-cultured with endothelial cells, release factors, at least in part by extracellular microvesicles (MVs), that are able to induce

endothelial cells to form a structure which resembles BBB (1). FGF2 and VEGF are some of the proteins contained in these structures (2-3). Vesicles released by oligodendroglioma (G26/24) cells contain pro-apoptotic factors, such as Fas Ligand and TRAIL (4-5), which could act synergistically in inducing brain cell death. In particular G26/24 MVs can inhibit neurite outgrowth, and induce apoptosis in about 75% of primary rat cortical neurons in culture and in 40% of astrocytes. We also demonstrated the horizontal transfer of labeled proteins mediated by vesicles among normal as well as tumor brain cells. Recent studies demonstrated that in cultured astrocytes, as previously found in developing rat brain, the amount of histone H1° increases during differentiation, while the level of its mRNA decreases, suggesting that its expression is mainly regulated at the post-transcriptional level (6). In contrast, G2624 maintain high levels of both H1° protein and mRNA, and release H1° protein into the culture medium by extracellular vesicles (8). These findings suggest that deregulation of H1° histone expression can be linked to tumorigenesis and oligodendroglioma cells can escape antiproliferative cues by discharging into the extracellular environment molecules expressed during differentiation, such as H1° histone.

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Identification of pathways involved in aneuploidy onset and its tolerance using a DNA microarray approach

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Keywords: **Aneuploidy, Bioinformatics, Biostatistics**

Genomic instability is a hallmark of the majority of human tumors explaining the heterogeneity shown by tumor cells. This phenomenon is often associated with chromosomal instability (CIN) and aneuploidy, a condition in which tumor cells lose or gain chromosomes. Previously, we showed that posttranscriptional silencing by RNAi of pRb[(1)], DNMT1[(2)] and MAD2[(3)] is associated with aneuploidy in cultured human cells reinforcing the idea that there are several roads leading to aneuploidy. In the attempt to understand if a common molecular signature exists that underlies aneuploidy and its tolerance in tumor cells, we did post transcriptional silencing of Rb, MAD2 and DNMT1 in human fibroblasts (IMR90) and analyzed their transcriptome by Microarray analysis. Using GeneSpring and the R-software for statistical analysis we identified a number of differentially expressed genes in the three samples analyzed when compared to the gene expression of the control. Some of the identified genes were differentially expressed simultaneously in at least two out of three samples analyzed. These data were analyzed using freeware bioinformatics software (DAVID, GOrilla) that showed the presence of a significant enrichment of genes involved in several biological process like G1/S transition, mitotic cell cycle, DNA Replication and DNA strand elongation.
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Transcriptional and proteomic analysis of p65(-1), a new isoform of the NF-kB complex.

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Keywords: **Infiammation, NF-kB, isoform, pIL-6-Luc, 2D elettroforesis**.

Nuclear Factor-κB (NF-κB) are ubiquitous transcription factors that in mammals regulate many biological process including inflammation, immunoregulation, apoptosis, cell growth and cell proliferation (1). NF-κB family members include *RelA* (p65), c-Rel, RelB, p50 and p52. These proteins exert their functions by binding as homodimers or heterodimers to specific DNA target sites (κB consensus). The p65/p50 heterodimer is the most abundant and investigated form of NF-κB (3). A new isoform of p65, named p65(-1), was discovered in human and mouse. This isoform contains an unknown exon (named exon –1) located upstream to the first known exon of *RelA*, coding for p65. Transcription of the exon -1 leads to an alternative splicing between exon -1 and exon 1, thus skipping exon 0. As consequence of this alternative splicing, p65(-1) lacks some amino acid residues belonging to the RHD. Our results show that p65(-1), compared to p65, has different biochemical properties in some cellular mechanism like transcriptional activity on kB consensus, apoptosis and regulation of the glucocorticoid receptor (GR) activation (4). We have analysed the transactivation of p65(-1) using natural promoter regions, linked with the pathway of NF-κB. The luciferase assays with pIL-6-Luc (interleukin 6) have been used for study the activity of p65(-1) and we have also analyzed p65(-1) activity with p65 or p50 under the same conditions. We have also studied p65(-1) expression on human peripheral blood mononuclear cells (PBMC) using 2D electrophoresis. Our data suggest that p65(-1) has a central role during the regulation of pro and anti-inflammatory responses through a specific transcriptional activation using different partners according to the cellular requirements.

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Regulation of the germline stem cells maintenance by the chromatin remodelling factor ISWI

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Keywords: stem cells, chromatin remodelling; Drosophila

The germline stem cells (GSCs) of *Drosophila melanogaster* ovary provide an excellent model system to study the molecular mechanisms of stem cell self-renewal. The balance between stem cell self-renewal and differentiation is precisely controlled in order to ensure tissue homeostasis and to prevent tumorigenesis. The regulation of stem cells maintenance and self-renewal depends on changes in chromatin organization and tissue-specific transcriptional regulators. In vivo studies have shown that in the Drosophila ovary the ATP-dependent chromatin-remodelling factor ISWI maintains germline stem cells in a single niche. However, the exact role of chromatin remodeling in stem cell niches is poorly understood.

To dissect the role of ISWI-mediated chromatin remodeler in controlling stem cell self-renewal, I developed a strategy to purify a large numbers of pure GSCs from the Drosophila ovary. Using this approach I generated a genome-wide transcriptome and chromatin-binding profile of ISWI on GSCs chromatin. To identify the potential regions of the genome that are bound by ISWI in GSCs, I conducted a ChIP-Seq analysis and found nearly 7000 ISWI bound coding genes. Moreover, RNA-Seq experiments conducted in ISWI mutant GSCs revealed ISWI as major regulator of about 70 % of its target genes in GSCs. Furthermore, by gene ontology analysis I identified specific gene networks under the control of ISWI. Particularly, I found that the ISWI regualtes genes playing an essential role in the maintenance of GSCs.

Our data suggest that the ATP-dependent chromatin remodeler ISWI works as a master regulator of GSCs self-renewal in the Drosophila ovary.

Reevaluating the function of a transcription factor: MBF-1 as a sea urchin chromatin organizer ?

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Keywords: **MBF-1 activator; CTCF; *Hox* genes; chromatin immunoprecipitation**

The Zinc-finger MBF-1 factor is involved in the expression of the early histone genes during development of the sea urchin embryo (1, 2). In spite of being a transcription activator, the DNA-binding domain of MBF-1 shares high sequence similarity with that of the chromatin organizer CTCF of vertebrates and drosophila (3). On the other hand, extensive *in silico* analysis failed to identify the sea urchin CTCF ortholog (4). This led us to speculate that MBF-1 somehow could have co-opted the function of CTCF during evolution of the echinoderms. Since in vertebrates CTCF binds *Hox* chromatin, to support our hypothesis, we first identified high-score putative binding sequences for CTCF/MBF-1 within the single sea urchin *Hox* gene cluster. Moreover, we observed the full evolutionary conservation of these binding sites in *S. purpuratus* and *P. lividus* species. Worth of mention, by chromatin immunoprecipitation (ChIP) assay, we detected the occupancy of MBF-1 on *hox11/13-a*, *-b*, and *-c* regulatory sequences at distinct stages of development. As expected from the binding of an activator, we found that the association of MBF-1 to the *cis*-regulatory sequences of both *hox11/13-a* and *-b* genes relates to the transcriptional status of these genes. Strikingly, we also mapped the physical binding of MBF-1 to *hox11/13-c*, which is instead not expressed during embryogenesis. Altogether, these observations indeed suggest the possibility that MBF-1, besides being a transcription activator, could also function as a general chromatin organizer. To further support this hypothesis, we are planning ChIP-seq experiments to identify the association of MBF-1 to the sea urchin chromatin at a genome-wide level.

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A sub-population of mesoangioblasts displays features of resistance and proliferation confirmed by transcriptome analysis

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Since all mammals mesoangioblasts (mabs) have the ability to proliferate in culture and to differentiate into various types of mesodermal cells, they are employed for cell-based therapies. These stem cells derive from dorsal aorta of embryos and are able to migrate during embryo development and also in postnatal life. To answer why stem cells die in few days after transplantation, we have planned a study in vitro subjecting the mouse mabs to a severe oxidative stress by H_2O_2. We have analyzed one of the cell clones derived from few survived cells and have found that it retains stemness profile (e.g. proliferation and differentiation) as that of mabs. Moreover, the clone's features are different from those of the rest of the mabs because after a second oxidative stress the clone is more resistant and does not block mitosis in the G_2/M phase of cell cycle. To solve if this clone is a sub-population we have performed the analysis of microarray assays and found genes differentially expressed in mabs and cell clone allowing a more precise phenotypic analysis.
About 800 significant (q<0.05 with FC±2) differentially expressed genes were found. Notably, in cell subpopulation a number of genes of the glutathione pathway (GSTa3, GSTM1, GST1-1, MGST1 and IDH1) were overexpressed. GSTs are enzymes that use glutathione as reducing oxidized compounds, and they also convert H_2O_2 to H_2O, while IDH1 has a significant role in NADPH production, by providing protection from ROS toxicity. This subpopulation is able to better eliminate ROS in comparison to the rest of treated mesoangioblasts and, thus, confirms its increased ability in ROS detoxification. Moreover, GSTM1 acts by sequestrating the ASK1 protein-kinase, which in turn cannot activate p38 MAPK involved in the mitosis blockade of mesoangioblast treated cells. The results suggest that the overexpression of these genes in the subpopulation corresponds to an increased capacity of detoxification and proliferation. The finding that mesoangioblast stem cell population is non-homogenous is very important for stem cell biology because it highlights a different capability, which is central in the development of cell-based therapies.

$p14^{ARF}$ prevents proliferation of aneuploid cells by inducing TP53-dependent apoptosis

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Keywords: **Mad2 haploinsufficiency, aneuploidy, Tp53, apoptosis, $p14^{ARF}$**

Abstract: Weakening the Spindle Assembly Checkpoint by reduced expression of its components induces chromosome instability and aneuploidy both hallmarks of cancer cells (1). The tumor suppressor $p14^{ARF}$ is overexpressed in response to oncogenic stimuli to stabilize p53 halting cell progression. Previously, we found that lack or reduced expression of $p14^{ARF}$ is involved in the maintenance of aneuploid cells in primary human cells suggesting that it could be part of a pathway controlling their proliferation (2). To investigate further this aspect $p14^{ARF}$ was ectopically expressed in HCT116 cells after depletion of the Spindle Assembly Checkpoint MAD2 protein that was used as a trigger for aneuploidy. $p14^{ARF}$ re-expression reduced the number of aneuploid cells in MAD2 post-transcriptionally silenced cells. Also aberrant mitosis frequently displayed in MAD2 depleted cells were decreased when $p14^{ARF}$ was expressed at the same time. In addition $p14^{ARF}$ ectopic expression in MAD2 depleted cells induced apoptosis accompanied by increased p53 protein levels. Conversely, $p14^{ARF}$ ectopic expression did not induce apoptosis in HCT116 p53KO cells. All together, our results suggest that the tumor suppressor $p14^{ARF}$ may have an important role to counteract proliferation of aneuploid cells by activating a p53-dependent apoptotis.

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Cationic SLN as Targeting Gene Delivery Systems For Hepatocellular Carcinoma

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Keywords: cationic SLN, gene delivery, hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third leading cause of cancer-related deaths. In the United States, the incidence of HCC has almost tripled during the past two decades and HCC has become one of the fastest growing cancers. While surgical removal of tumor tissues is an effective approach to protect relatively healthy liver tissue, it is only applicable to a small subset of HCC patients with specific pathological conditions, such as confined tumor mass without portal hypertension. Therefore, there is an urgent need to develop novel therapeutic strategies to treat this deadly disease. Systemic tumor-targeted gene delivery is attracting increasing attention as a promising alternative to conventional therapeutic strategies. At this purpose a large number of viral and non-viral vectors have been studied and applied as systems of stable transfection with low toxicity. Although cationic polymers and liposome are promising systems, solid lipid nanoparticles (SLN) have been recently proved to be a really useful vehicle for gene therapy [1,2].

The aim of this work was to design and to obtain cationic SLNs capable of forming complexes with siRNA and DNA plasmid for the treatment of HCC. The physical binding between cSLN and nucleic acids was confirmed by the study of complexes' zeta potential values that became more positive as higher was the amount of cSLN and via the electrophoretic mobility of the samples in agarose gel 0.8%. Transfection studies on different tumor cell line are in progress.

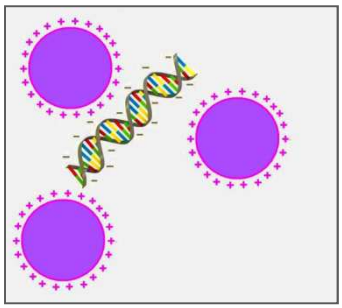

Figure 1. Cationic SLN-DNA interaction.

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The role of side chains in Substituted Pyrrole derivatives towards antiproliferative activity

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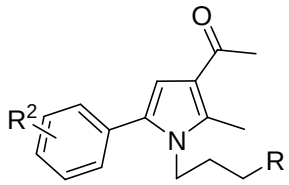
Keywords: **Anticancer agents, Pyrrole derivatives, Side Chain**

In the last years, the introduction of side chains in different molecular compounds takes increasing importance. As recently reported in literature, heterocyclic scaffolds with poor biological activity , if properly decorated with selected side chains, can improve their anticancer activity against a large spectrum of human tumor cell lines. For example, the annelated Pyrrolo[3,4-e]Pyrimidines and Pyrrolo[3,2-e]Pyrimidines, opportunely functionalized with a large number of side chains, showed a good increase in the antitumor activity with respect to the starting core structure. New compound thus obtained showed antiproliferative activity against all the human tumor cells, generally in the low micromolar concentration range (1).
Furthermore, this increase in activity is confirmed in the new linear derivatives Thieno[3,2-d][1,2,3]Triazolo [1,5- α]Pyrimidines (2) and their angular isomer Thieno[2,3-e][1,2,3]Triazolo[1,5- α]Pyrimidines (3).
Another confirmation is provided by the Indolo [3,2-e][1,2,3]Triazolo [1,5- α]Pyrimidines, that resulted inactive as antitumor agent, but when a functionalization with side chains takes place, the anticancer activity has interestingly appeared (4).



Here, with the aim to explore the importance of the addition of side chains on smaller molecular scaffolds, of easier synthetic access and decoration, we focused our attention on a new series of substituted "chained" pyrrole derivatives in order to evaluate their anticancer potential.

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Indice Autori presentazioni orali:

Indice Keywords:

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ISBN 9788890580550

ISBN 9788890580550