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INTERDISCIPLINARE
TRASLAZIONALE
IN AMBITO BIOMEDICO

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BIOINFORMATICA IMMUNOLOGIA
MALATTIE APPARATO RESPIRATORIO
MALATTIE METABOLICHE
MICROORGANISMI NELLE BIOTECNOLOGIE
NANOTECNOLOGIE NEUROSCIENZE
ONCOLOGIA SVILUPPO E DIFFERENZIAMENTO

LIBRO degli ABSTRACT

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Progetto grafico:
Anna Bonomolo (IBIM-CNR)

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

ON8
A biclustering approach for the analysis of miRNA expression profiles

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

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

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

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

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

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

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

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

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