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

LIBRO degli ABSTRACT

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

ON3

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

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

ON4

S100 proteins in breast cancer: multiomics-based analyses

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

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

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

ON5

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

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