duced from both normal and cancer cells, malignant cells release a much higher amount of EVs, which also contain tumor-specific proteins and RNAs.
We previously found that G26/24 oligodendroglioma cells shed EVs that contain the pro-apoptotic factors FasL and TRAIL[1,2]. Interestingly, G26/24 release, via EVs, extracellular matrix remodelling proteases[3], and H1° histone protein[4], and mRNA. To shed further light on the role of EVs in discarding proteins and mRNAs otherwise able to counteract proliferative signals, we studied a melanoma cell line (A375). We found that also these cancer cells produce H1° and release it into the extracellular space by EVs. Interestingly, H1° sorted to vesicles has a molecular mass higher than expected, and is probably sumoylated. By T1 RNase-protection assay with the H1° RNA, three main complexes were evidenced in EVs, the most abundant of which has a molecular mass of about 65 kDa. By using a biotinylated H1° RNA to fish interacting factors, we isolated from EVs a few proteins which have been then identified by mass spectrometry: the most abundant is a protein of about 60 kDa: myelin expression factor-2 (MYEF2). Western blot analyses confirmed the presence of MYEF2 in EVs released from A375 melanoma cells.

1. D'Agostino S et al. Int J Oncol 2006, 29:1075-85.
2. Lo Cicero A et al. Int J Oncol 2011, 39:1353-57.
3. Lo Cicero A et al. Matrix Biol 2012, 31:229-33.
4. Schiera G et al. Int J Oncol 2013, 43:1771-76.

DECORIN EFFECTS ON PROTEOMIC PROFILING OF BREAST CANCER CELLS: AN UPDATED STUDY.

G. Di Cara[1], I. Pucci-Minafra[1], P. Cancemi[1,2], G. Contorno[1], A. Forlino[3], M.E. Tira[4], R. Tenni[3], D. Martini[5], S. Minafra[1]

[1]Centro di OncoBiologia Sperimentale, University of Palermo, La Maddalena Cancer Center, Palermo, Italy; [2]Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, [3]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [4]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy; 5Department of Human Anatomical Sciences and Physiopathology of Locomotor Apparatus, Human Anatomy Section, University of Bologna, Bologna, Italy

The malignant carcinomas are characterized by several capabilities acquired by the neoplastic cells, among which the ability to invade the extracellular matrix (ECM) and to establish a crosstalk with several ECM components.
Under this respect, the extracellular microenvironment is an entity extraordinarily rich of information with opposite signals. Our group has long undertaken the study of the effects of ECM molecules on the behavior of cancer cells in vitro. Among the studied molecules, the decorin was found to exert a non-permissive effect on the growth and motility of the transfected tumor cells. The decorin, belongs to the family of small leucine-rich proteoglycans (SLRP) and is involved physiologically in the fibrillogenesis of collagen. In the last few year, a new anti-oncogenic role has been proposed for decorin[1].
This study aimed to implement the knowledge on the effects of ectopic decorin on breast cancer cells, using as a reference point the results already achieved by our research group[2] on the experimental model format. By breast cancer cell line 8701-BC and its transfected clone DEC-C2.
The extension of the proteomic analysis combined with the mass spectrometry, allowed to triplicate the number of identified proteins in our model. Among the newly identified proteins were members of the classes of metabolic enzymes, S100 family and cell motility proteins, which revealed a net decrease in the decorin transfected cells. Of considerable importance is the observation that these classes of proteins are the most involved in metastatic progression. These results confirm and reinforce the anti-oncogenic role hypothesized for decorin.
The work was co-funded by the Italian 5x1000 to COBS.

1. Santra M et al. J Biol Chem 2000, 275:35153-33161.
2. Pucci-Minafra I et al. Connect Tissue Res 2008, 49:30-41.

HIGH MOLECOLAR WEIGHT HYALURONAN MODULATES SERGLYCIN-MEDIATED CD44 ACTIVATION IN CHONDROCYTE CULTURES STIMULATED WITH IL-1β

M. Scuruchi[1], A. Avenoso[2], A. D'Ascola[2], A. Calatroni[2], S. Campo[2], G.M. Campo[1]

[1]Department of Experimental and Clinical Medicine and [2]Department of Biomedical Sciences and Morphological and Functional Images, University of Messina, Messina, Italy

E-mail: mscuruchi@unime.it

Serglycin is a widely distributed proteoglycan, previously assumed to be hematopoietic cell specific. However, during the last decade, numerous studies have demonstrated that such PG is also synthetized by various non-hematopoietic cell types and it is involved in a plethora of both normal and pathological conditions. Serglycin secretion could be induced in several cell types upon external inflammatory stimulation. The biosynthesis of serglycin is up-regulated by lipopolisaccaride (LPS) in macrophages and in primary human endothelial cells, by tumor necrosis factor (TNF) in endothelial cells and adipocytes, as well as by IL-1β in smooth muscle cells. Our data show that serglycin is also synthetized in primary human chondrocytes following stimulation with IL-1β. Since serglycin has been shown to be a ligand for hyaluronan receptor CD44 and such interaction could amplify inflammatory process, we decided to evaluate the mRNA and protein levels of CD44 after IL-1β administration in chondrocytes. The stimulation of cells with IL-1β resulted in an increase of both serglycin and CD44 mRNA and protein expression. Therefore, we observed a significant increase of pro-inflammatory cytokines, such as TNF-α and IL-6. These results suggest that serglycin, as well as CD44, could participate in the inflammatory process of chondrocytes. To further analyze the importance of serglycin during inflammatory response in human chondrocytes we treated such cells with a serglycin siRNA in order to block its production. Blocking serglycin production caused a significant reduction of CD44, as well as of the pro-inflammatory cytokines levels, indicating that the serglycin, released following IL-1β stimulation , is able to increase inflammation by modulating CD44 activity in human chondrocytes. The treatment with high molecular weight hyaluronan (HMWHA), after IL-1β administration, induced a further decrease in pro- inflammatory cyotokines and also a reduction of serglycin production. The effect was more marked when both serglycin siRNA and HMWHA were added together. Such results, taken together, suggest that this proteoglycan is able to modulate inflammation via CD44 receptor. In conclusion, we believe that the serglycin pathways should be carefully considered for future anti-inflammatory strategies although further studied are needed to fully confirm these findings.

1. Kulseth MA et al. Biochim Biophys Acta 1999, 1428:225-32.
2. Scully OJ et al. Anat Rec 2012, 295:1415-420.
3. Korpetinou A et al. Front Oncol 2014, 3; 327.
4. Trine MR et al. Biochim Biophys Acta 2014, 1840:2498-505.