(APE) all in suboptimal concentrations, on the viability of MDA-MB231 and MDA-MB468 tumors measured with by the MTT reagent. We observed that the combination of PX ($10^{-8}/10^{-9}$ M) + Carb ($10^{-12}/10^{-10}$ M) reduces the viability of MDA-MB231 and MDA-MB468 cells (36.8 ± 6.2; p<0.001 and 33.4 ± 2.5; p<0.001 respectively). When Carb was replaced by the selective M2 agonist APE ($10^{-5}/10^{-7}$ M), there was also a significant decrement in cell viability (MDA-MB231: 35.8 ± 3.17; p<0.001; MDA-MB468: 26.9 ± 3.6; p<0.001). The effects produced by the combination containing Carb or APE were blocked in the presence of AT (10^{-7} M) or methoctramine (MET; 10^{-5} M) (non-selective or M2 selective antagonists respectively). Similar results were obtained when DOXO was employed instead of PX in the combination (DOXO (10^{-8} M) + Carb: MDA-MB231: 35.3 ± 0.8; p<0.001 and MDA-MB468: 21.1 ± 0.7; p<0.01. DOXO (10^{-8} M) + APE: MDA-MB231: 33.3 ± 2.1; p<0.001 and MDA-MB468: 31.2 ± 0.9; p<0.01). The observed effects were inhibited in the presence of AT or MET respectively. We conclude that the combination of a conventional cytotoxic drug with a muscarinic agonist is useful to reduce the viability of triple negative tumor cells, which could be a new form of treatment focused on mAChRs for these tumors.

0151 - AUTOPHAGY MODULATES THE IMMUNE RESPONSE OF PANCREATIC TUMOR CELLS BY CONDITIONING THE EXOSOME COMPOSITION.

Daniela Laura PAPADEMETRIO | Nadia PAGLILLA | Estefania PAPARATTO | Daniel Hector GRASSO | **Maria Noé GARCIA** | Elida ALVAREZ

INSTITUTO DE ESTUDIOS DE LA INMUNIDAD HUMORAL (IDEHU) - FFYB-UBA-CONICET

Abstract/Resumen: Pancreatic ductal adenocarcinoma (PDAC) is characterized by inducing immunotolerance, where exosomes act as intercellular messengers, carrying molecules from the tumor cells to the immune cells. In this work we investigated the role of autophagy in the composition of tumor-derived exosomes and their impact on the activity of Dendritic (DC) and Natural Killer cells (NK). For the experiments we used two PDAC cell lines, MIAPaCa-2 and PANC-1, and two inhibitors of autophagy, 3-Methyladenine (3-MA) and Spautin-1 (SP-1). First, we demonstrated the presence of exosomes in culture cells supernatants with or without 3-MA or SP-1 by electron microscopy. Interestingly, both treatments also increased the exosomal marker CD63 observed by WB. Afterward, monocyte-derived-dendritic-cells (MDDC) were treated with the different populations of exosomes and after 1 h LPS. Cytokine production by ELISA was evaluated in 48 h supernatant. MDDCs incubated with exosomes from cell culture without SP-1 secreted TGF-ß, meanwhile the exosomes from cells with SP-1 induced the secretion of IL-12, and increment in HLA-DR expression on MDDC membrane (observed by flow Cytometry) (p<0.01). No differences were observed in IL-10 profile. NK cytotoxic activity was evaluated in K562 cell line. We incubated NK cells with exosomes from supernatant of MIAPaCa-2 and PANC-1 cells treated or not with SP-1, for 2-6 h. After CFSE staining of K562 cells, co-cultures of NK:K562 (ratio 5:1) were performed for 4h. Cytotoxicity of NK was evaluated by CFSE/PI stain. Exosomes from SP-1 treated cell supernatant stimulated cytotoxic activity of NK cells (p<0.05). Moreover, this treatment increased the IFNg production by NK cells (p<0.01). Our results suggest that autophagy condition exosome-composition, activating NK activity but inducing a tolerogenic profile in DC. Furthermore, we speculate that autophagy pathway status in cancer cell may modulate the immune tumor microenvironment through the exosome profile composition.

0153 - THE TREATMENT OF MCF-7 CELLS WITH CARBACHOL AND PACLITAXEL IS EFFECTIVE TO REDUCE TUMOR CELL GROWTH IN VITRO AND ANGIOGENESIS IN VIVO.

Agustina SALEM | Paola MARTINEZ PULIDO | Yamila SANCHEZ | Francisco SANCHEZ | Alejandro Javier ESPAÑOL | María Elena SALES

CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA

Abstract/Resumen: Previously we demonstrated that muscarinic receptors (MR) are expressed in different types of human and murine breast tumors. Their activation with the synthetic agonist carbachol (Carb) promotes cell death and improves the effect of paclitaxel (PX), a cytotoxic drug commonly used in breast cancer treatment. In this work, we analyzed the ability of a combination of low concentrations of Carb+PX, simulating a metronomic schedule, to reduce cell growth in bi (2D) and tridimensional (3D) MCF-7 cell cultures (by MTT assay and by microscopy respectively). We also studied the effect of this combination in HMEC-1 cells´ tubulogenesis and the in vivo effect on the neovascular response (Nº vessels/mm^2) in mice tumor bearers. We observed that the treatment of MCF-7 cell spheroids with Carb (10^{-11} M) + PX (10^{-9} M) significantly reduced their 3D growth compared to control spheroids by 33 ± 3 % at day 6 of culture (p<0.01). In addition, Carb+PX significantly decreased HMEC-1 cells tubulogenesis (55 ± 7 %; p<0.01). The administration of two cycles of subtherapeutic doses of Carb+PX to tumor bearer mice, diminished the neovascular response produced by MCF-7 cells (MCF-7: 3.9 ± 0.3; MCF-7+Carb+PX: 3.1 ± 0.3; p<0.0001). The previous treatment with the antagonist atropine reverted the effect produced by the combination (4.2 ± 0.2 %).Interestingly, the administration of PX at therapeutical doses increased the neovascular response produced by MCF-7 cells (4.4 ± 0.4; p<0.001). Our results demonstrate that the combination of Carb+PX has more specificity than conventional chemotherapy, since it targets MR and it has an anti-angiogenic effect not seen with the cytotoxic drug at therapuetical doses.

0154 - DIFFERENTIAL ROLE OF AHCYL1 GENE IN TUMOR PLASTICITY

Nicolàs BUDNIK (1) | Melina MUÑOZ BERNART(1) | Malena MANZI(2) | Maria Eugenia MONGE(2) | Joaquín M. ESPINOSA(3) | Gustavo MOSTOSLAVSKY(4) | Carolina PEREZ CASTRO(1)

INSTITUTO DE INVESTIGACIÓN EN BIOMEDICINA DE BUENOS AIRES (IBIOBA) - CONICET (1); CENTRO DE INVESTIGACIONES EN BIONANOCIENCIAS (CIBION), CONSEJO NACIONAL DE INVESTIGACIONES (2); LINDA CRNIC INSTITUTE FOR DOWN SYNDROME, UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS (3); CENTER FOR REGENERATIVE MEDICINE (CREM), AND SECTION OF GASTROENTEROLOGY, DEPARTMENT OF MEDICINE, BU (4)

Abstract/Resumen: Malignant reprogramming of cells is responsible for tumor development. During this process stem-like tumor cells that acquired self-renew capacity produce heterogeneity, tumor dissemination, and relapse after cancer therapy. We have previously identified AHCYL1 as a potential regulation target of core transcription factors OCT-4, SOX-2, and NANOG responsible for cell reprogramming. We studied AHCYL1 by analyzing its cellular location and expression levels during cell plasticity events of tumor cells. We used the glioblastoma (GBM) cell line U87 and lung carcinoma (LC) cell line H1299 as in vitro models since brain and lung have the highest Ahcyl1 expression. We cultured these cell lines in a 3D format in DMEM/F12 medium supplied with FGF, EGF, and B27 and compared with 2D format cultured cells with DMEM serum complemented medium. Ahcy1 localization was determined by immunofluorescence assay and cell fractioning followed by Western blotting. To generate U87 and H1299 Ahcyl1 knockdown stable lines, three different shRNAs were tested and the expression levels of Ahcyl1 and the core factors were determined by Western blot and RT-qPCR. Stemness potency was evaluated by ELDA assay (extreme limiting dilution analysis). We found that AHCYL1 localizes both in nuclei and cytosol, in addition to a putative processed isoform in nuclei. In 3D cultures, Ahcyl1 expression is differently