(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

regulated compared to 2D cultures. Also, in GBM, AHCYL1 expression was significantly increased, in contrast in LC was decreased ($p<0.0001$ and $p<0.05$ respectively). In Ahcyl1-depleted LC cells, the core factors expression levels and the stem potency were increased ($p<0.05$). Altogether, we conclude Ahcyl1 has a key role as a regulator of stem potency and would be dependent on tumor type.
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0186 - INHIBITION OF BREAST TUMOR GROWTH BY N(G)-NITRO-L-ARGININE METHYL ESTER (L-NAME) IS ACCOMPANIED BY ACTIVATION OF FIBROBLASTS

Marianela SCIACCA | Denise BELGOROSKY | Macarena ZAMBRANO | José Ignacio GOMÉZ ESCALANTE | Fernanda ROCA | Yanina Verónica LANGLE | Eduardo O. SANDES | Catalina LODILLINSKY | Ana Maria EIJAN

INSTITUTO DE ONCOLOGÍA ANGEL H. ROFFO

Abstract/Resumen: Nitric Oxide (NO) is generated by a family of NO synthases (NOS), being the inducible isoform (iNOS) which produces higher NO levels. This, often acts as a survival factor, hence inhibition of iNOS has been proposed as a targeted therapy. Fibroblasts, the main cell type in tumor microenvironment, have been described as a heterogenic population and their role in breast cancer associated to NO inhibition has not been yet elucidated. In this work we use murine and human breast cancer cell lines to evaluate the impact of NO inhibition in tumor progression. LM3 and its more aggressive variant LMM3 cell line expressed iNOS, as well as the human MDA-MB-231 cells (qPCR $p<0.05$; $p<0.001$). On the other hand, LM2 and human MCF10DCIS.com, line did not express iNOS. Inhibition of NO production by L-NAME abrogates viability and treatments with the NO-donor, DETA/NO, induced cell viability only in iNOS positive cancer cells (MTS assay $p<0.05$; $p<0.01$; $p<0.001$). L-NAME abrogates ERK activated signalling pathways only in iNOS positive human and murine cancer cell lines (Wenstern blot $p<0.05$; $p<0.01$). In vivo, L-NAME inhibited tumor growth in iNOS positive cells ($p<0.001$ vs. CRL). In pararallel, collagen deposition and α-SMA positive stromal cells was observed. In iNOS negative cells, no effect on viability, ERK activation and tumor size reduction was observed with L-NAME. On the other hand, L-NAME induces an opposite effect on fibroblast, showing an increase in viability and differentiation. In contrast, DETA-NO reduced their viability (MTS assay $p<0.05$). Our results reveal that NO inhibition contributes to stimulate proliferation and activation of fibroblasts in parallel with tumor reduction only in iNOS positive breast cancer. Hence, iNOS inhibition could be considered as new therapeutic targets to be added to conventional therapies.

0502 - CHANGES IN APOPTOSIS LEVELS ARE ASSOCIATED WITH HYPOXIA-MEDIATED TRASTUZUMAB AND T-DM1 RESISTANCE IN HER2+ BREAST CANCER

Virginia Judith WOLOS | Ivana J. TAPIA | Marianela ABRIGO | Elisa D. BAL DE KIER JOFFÉ | Gabriel FISZMAN

UNIVERSIDAD DE BUENOS AIRES, INSTITUTO DE ONCOLOGÍA "ÁNGEL H. ROFFO"

Abstract/Resumen: Trastuzumab and trastuzumab emtansine (T-DM1) immunotherapies are the treatment of choice for HER2+ breast cancer patients. However, although their success, de novo or acquired resistance is still the main obstacle in clinical practice. Our previous studies demonstrated that hypoxic microenvironment promotes trastuzumab and T-DM1 resistance in HER2+ breast cancer cells. In this work we further analyzed mechanisms determining hypoxia-induced resistance. First, we confirmed in concentration-response curves the significant differences between normoxic and hypoxic conditions with (1 - 50) µg/mL trastuzumab and (0.1 - 10) µg/mL T-DM1 three-day treatments on BT-474 cell line ($p<0.001$). In order to determine whether it was due to a reduction of cell viability or to a modulation of cell cycle, we performed flow cytometry analyses. Interestingly, we observed that hypoxic conditions reduced trastuzumab and T-DM1-mediated apoptosis ($p<0.05$). In contrast, there were no significant differences between drug effects on the cell cycle either under normoxic or hypoxic conditions. Since modulation in the HER2 expression is associated not only with trastuzumab mechanisms of action but also with drug resistance, we asked if hypoxia regulated BT-474 HER2 levels in response to drug treatment. Further flow cytometry analyses showed that trastuzumab and T-DM1 decreased HER2 expression on cell surface ($p<0.05$) regardless of hypoxic conditions. In summary, our results show that lower levels of apoptosis under hypoxia mediate trastuzumab and T-DM1 resistance. However, the question of the mechanism underlying this effect is still open. In our laboratory, by mammosphere assay, we observed that hypoxic BT-474 cells developed a higher proportion of breast cancer stem cells than normoxic cells. These results highlight an increase in the breast cancer stem population as a potential mechanism of hypoxia-mediated trastuzumab and T-DM1 resistance, which deserves to be studied.

0711 - RANITIDINE HINDERS RADIATION INDUCED MESENCHYMAL TRAITS IN EXPERIMENTAL PANCREATIC ADENOCARCINOMA

Nora A MOHAMAD | Tamara E GALARZA | Graciela P CRICCO | Gabriela A MARTIN

LABORATORIO DE RADIOISÓTOPOS, FACULTAD DE FARMACIA Y BIOQUÍMICA, UBA

Abstract/Resumen: We have previously set that the antihistamine ranitidine (R) hindered growth of human pancreatic PANC-1 and BxPC3 grafts and the development of PANC-1 lung metastasis in nude mice. Two Gy irradiation increased PANC-1 tumor growth while slowered BxPC3, though increased lung metastasis in both. R reduced irradiated tumor growth rate and lung metastases. The aim of this work was to evaluate the effect of irradiation and R on epithelial to mesenchymal transition (EMT), a process associated with invasion and metastasis, in pancreatic tumors. Dedifferentiated PANC-1 and more differentiated BxPC3 tumors were irradiated (I) or not (C) with 2 Gy of gamma radiation, transplanted to non-irradiated mice, and treated with R 150 mg/kg.day, p.o. (I+R; C+R) or not (I; C). Immunohistochemistry was performed to evaluate the expression of EMT molecular markers (E-cadherin, vimentin, Slug) and of TGF-ß1 (a major promoter of EMT). In PANC-1 tumors epithelial marker E-cadherin was not detected in any group, while transcription factor Slug nuclear expression was similar in all of them. In C-grafts we observed a big number of vimentin (mesenchymal marker) and TGF-ß1 positive cells that was even bigger in I-tumors ($p<0.05$), but not in R and I+R. In BxPC3 only I-tumors did not show E-cadherin at cell membrane in the inner areas of slices. Very few cells expressed vimentin and TGF-ß1 in C-tumors; this expression was enhanced in I-group ($p<0.01$) but not changed in I+R or R. An increase in nuclear Slug and TGF-ß1 was detected only in I-grafts ($p<0.05$). TGF-ß1 correlated positively with vimentin in both tumor types ($p<0.01$). Nuclear Slug positively correlated with vimentin and TGF-ß1 only in BxPC3 ($p<0.01$). In vitro studies showed an increase in vimentin expression and cell migration in both irradiated cell lines that was blocked by R. In conclusion, R could reduce radio-induced gain of mesenchymal features, pointing out the relevance of research on drugs that control both growth and metastasis.

Genética / Genetic

Chairs: Florencia Giliberto/ Ariel López