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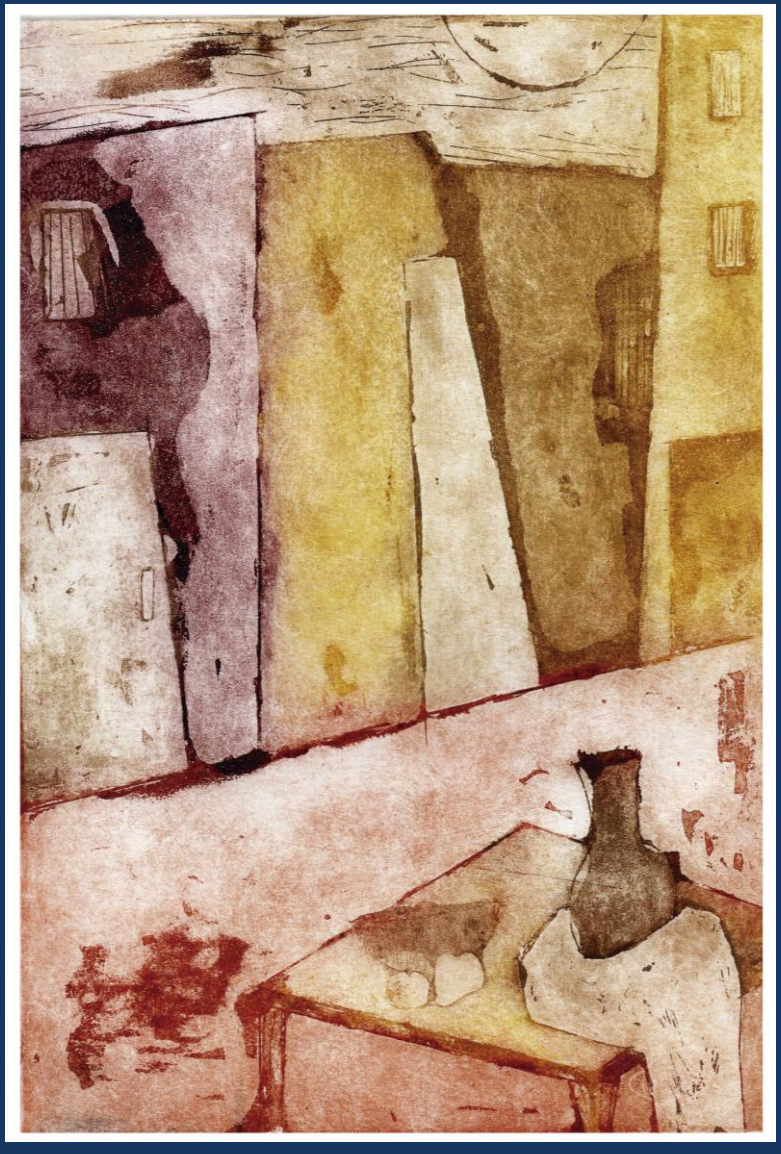

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La Tapa (Ver pág. 4)
Atardecer en la tarde
Antonella Ricagni

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(NANOMED-ar)

VI Reunión Científica Regional de la Asociación Argentina de Ciencia y Tecnología de Animales de Laboratorio (AACyTAL)

con la participación de
The Histochemical Society

13 - 16 de noviembre de 2019
Hotel 13 de Julio - Mar del Plata

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VI Regional Scientific Meeting of Asociación Argentina de Ciencia y Tecnología de Animales de Laboratorio (AACyTAL)

with the participation of
The Histochemical Society

November 13th – 16th, 2019
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Abstract/Resumen: Bladder cancer (BCa) is one of the most common tumors of the male urogenital tract and an important cause of death. The treatment of the invasive tumor is radical cystectomy. Currently, to improve patient's standard of living, conservative surgery followed by chemo (CT) and radiotherapy is proposed. Cancer Stem Cells (CSC) are a minority cell population associated with treatment resistance and tumor recurrence, so their quantification and identification are of special interest. Lab on a Chip (LOC) systems, have surged, in the last decade, as a powerful tool for cell individual study, with the benefit of using a low amount of biological samples. Our objective was to quantify and evaluate pluripotency and tumorigenic capacity of CSC in MB49-I, an invasive BCa cell line, post-treatment with CT agents, in macro culture conditions and in LOC systems. Doxorubicin (Doxo) or cisplatin (CisPt) treatment decreased the number of CSCs, measured as sphere formation efficiency ($p<0.05$) and decreased the remaining cells survival by 90% with cisPt and 65% with doxo ($p<0.001$). The histological study revealed that both CT generate smaller, disintegrated and eosinophilic spheres regard to control. Doxo or cisPt treatment induced an increase of pluripotential markers Oct-4, Sox2 and Nanog by qPCR ($p<0.005$ vs control). The in vivo growth of 15000 cells/mouse, derivate from spheres showed tumorigenic capacity, which could be responsible for tumor recurrence. Conclusion: The determination of the number of CSC, determinated as spheres, could be taken as a predictive marker of treatment response. The use of LOC devices provide the additional advantage of evaluating small samples, with translational possibility in patients.

0321 - RELEVANCE OF NITRIC OXIDE IN GLIOBLASTOMA THERAPY

Elsa Lourdes HINCAPIÉ ARIAS | Yanina Verónica LANGLE | Eduardo Omar SANDES | Denise BELGOROSKY | Ana Maria EIJAN

INSTITUTO DE ONCOLOGÍA ANGEL H. ROFFO

Abstract/Resumen: Glioblastoma (GBM) is the most common primary brain tumor, and within gliomas, the one with highest malignancy. Temozolomide (TMZ) treatment followed by radiotherapy is the main therapeutic strategy. Despite aggressive treatment, the clinical evolution ends in relapses with a live expectancy of less than two years. Increasing evidence associates the capacity of tumor regeneration and metastases with the presence of cancer stem cells (CSC). Furthermore, it has been suggested that nitric oxide (NO), as a consequence of the expression of inducible NO synthase (iNOS) could benefit GBM growth, progression and CSC maintenance. The objective of this study was to evaluate the role of iNOS in human GBM cell line U87, using specific pharmacological inhibitors (1400W, S-methylisothiourea (SMT)) alone and in combination with TMZ. Cell viability was evaluated by MTS assay after 5 days of treatment. Number of CSC was determined as sphere forming efficiency (SFE), in low attachment conditions supplemented with B27 in absence of fetal serum bovine. In 2D SMT and 1400 W (10 and 50 µM) did not affect U87 viability. TMZ (500 µM) alone or combined with iNOS inhibitors (50 µM) reduced 50 % cell viability ($p<0.05$). Regarding CSC, it was observed that iNOS inhibitors (25 µm) decreased 50 % SFE ($p<0.01$). TMZ alone (100, 250 µM) was able to inhibited 25 and 63 % SFE respectively ($p<0.01$), while the effect was even higher when 250 µM TMZ was combined with 25 µM SMT, reducing 78 % the SFE ($p<0.001$). These results shows that inhibition of NO through pharmacological iNOS inhibitors could be a potential therapeutic target in GBM since reduced U87 cell growth in 2D and affected stem cell compartment, described as responsible of tumor recurrence. These results suggest that NO inhibitors could be considered useful to be combined with conventional therapy in order to reduce disease relapses. Even though, more investigation in this field is needed.

0379 - DRUG REPURPOSING OF ß-BLOCKER PROPRANOLOL IN OSTEOSARCOMA: PRECLINICAL ANTITUMOR EFFICACY ON 2D/3D CELL GROWTH, CHEMOTAXIS AND XENOGRAFT PROGRESSION, ALONE OR IN COMBINATION WITH STANDARD-OF-CARE CHEMOTHERAPY

Luisina María SOLERNÓ | Natasha SOBOL | Rocio Belen RODRIGUEZ | Marina PIFANO | Giselle V. RIPOLL | Daniel Fernando ALONSO | Juan GARONA

LABORATORIO DE ONCOLOGÍA MOLECULAR, UNIVERSIDAD NACIONAL DE QUILMES

Abstract/Resumen: Osteosarcoma (OS), a bone cancer which primarily affects adolescents and young adults, is considered a clinical challenge due to its aggressiveness, rapid progression and limited response to standard of care therapies (SoC) such as cisplatin and methotrexate. Propranolol (PPN) is a non-selective ß-adrenergic receptor (ß-AR) antagonist originally used in the treatment of diverse heart diseases. Given that ß-AR signalling regulates many cellular processes involved in the initiation and progression of cancer, multiple efforts have been made to repurpose PPN in indications such as breast cancer, melanoma and angiosarcoma. Considering the unsatisfied clinical needs of OS, the objective of this work was to evaluate the in vitro/in vivo antitumoral activity of PPN as a monotherapy and/or in combination with SoC chemotherapy, in highly aggressive MG-63 and U-2OS human OS cells. PPN blocked promitogenic ß-AR activation by catecholamines in OS cells and drastically reduced clonogenic growth, chemotaxis and proliferation of exponentially-growing OS cells ($IC50= 45$ µM; $p<0.001$). Furthermore, 3D tumor spheroid growth was completely inhibited by PPN after a 7-day treatment. Synergistic cytostatic effects ($CI <1$) were observed after combining PPN (10 and 50 µM) with different optimal and suboptimal concentrations of cisplatin or methotrexate for 72 h. In animals bearing growing OS s.c. xenografts sustained treatment during 4 weeks with PPN (10 mg/kg i.p. daily), alone or in addition to cisplatin (suboptimal dose of 2 mg/kg i.p., three times per week), markedly abrogated tumor progression, exhibiting modulation of local tumor aggressiveness and reducing tumor growth rates by 25 or 75 %, respectively ($p<0.01$). Cisplatin treatment alone failed to inhibit OS xenograft growth. Conclusions. PPN showed a robust antitumoral activity alone or in combination with SoC chemotherapy in different OS preclinical models.

0406 - C-TERMINAL-TRUNCATED AND FULL LENGTH HEMEOXYGENASE-1 EXERT OPPOSITE BEHAVIOR OF HEAD AND NECK CANCER CELLS

Marilina MASCARÓ (1) | Exequiel Gonzalo ALONSO(1) | Norberto Ariel GANDINI(1) | Maria Julia FERRONATO(1) | Josefina Alejandra GUEVARA(1) | Eliana Noelia ALONSO(1) | Georgina Pamela COLÓ(1) | Pamela PICHEL(2) | Sergio Ceferino RECIO(2) | Alejandro Carlos CURINO(1) | Maria Marta FACCHINETTI(1)

INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (1); HOSPITAL MUNICIPAL DE AGUDOS "DR. L. LUCERO" (2)

Abstract/Resumen: We previously reported that heme oxygenase-1 (HO-1) protein is up-regulated in human HNSCC samples and that it is localized in the cytoplasmic and nuclear compartments. We also reported that high expression of HO-1 mRNA is associated with worst survival and that pharmacological activation of HO-1 by hemin increases viability of HN13 cells. However, how full length (FL-HO1) and C-terminal truncated (t-HO1) HO-1 affects HNSCC remains elusive. In this study, we aim

to elucidate if such forms of HO-1 impacts on head and neck cancer cells behavior. We established the FL-HO1 and t-HO1 overexpressing HN13 cells. We evaluated cell viability by crystal violet method, cell cycle progression by propidium iodide staining and flow cytometry, and cell migration by wound healing assay. In addition to our previous results using hemin, we found that 80 µM hemin increased cell number in S- ($p<0.001$) and G2/M ($p<0.001$) phases and diminished cell number in Go/G1 phase ($p<0.001$) at 72h. We also found that hemin delayed cell migration ($p<0.01$) respect to control. On the contrary, at same conditions, hemin failed to increase cell viability ($p>0.05$) neither alters cell cycle progression ($p>0.05$) in the normal keratinocyte cell line, HaCaT. By a genetic approach, we found that FL-HO1 HN13 cells have a higher growth rate ($p<0.001$) than its control and cell cycle progression is as similar as ($p<0,001$ vs control) it was observed with hemin treatment. However, FL-HO1 failed to alter migratory capacity ($p>0.05$). We also found that t-HO1 expression impaired HN13 cell viability ($p<0.01$ vs. FL-HO1 HN13) and induces a Go/G1 arrest ($p<0.01$) and a diminished cell number in SubGo ($p<0.01$) and S- ($p<0.05$) phases. Also, we found that t-HO1 expression delayed cell migration ($p<0.001$) respect to FL-HO1 HN13. In conclusion, our results show that head and neck cancer cells survival, cell cycle progression and migration capacity depends on predominant HO-1 form.

0407 - NOVEL CALCITRIOL ANALOGUES EM1 AND UVB1 AGAINST AGGRESSIVE BREAST CANCER CELLS AS A MONOTHERAPY OR IN COMBINATION WITH PACLITAXEL.

Josefina Alejandra GUEVARA (1) | Giuliana PAOLILLO(1) | Agustina IBARRA(1) | Eliana Noelia ALONSO(1) | Enrique Javier ARENAS LAHUERTA(2) | Mercedes NADAL SERRANO(2) | Joaquín ARRIBAS(2) | Cristina BERNADÓ MORALES(2) | Yagamare FALL(3) | Evangelina MASCARÓ(4) | Cristian VITALE(4) | Alejandro Carlos CURINO(1) | María Marta FACCHINETTI(1) | María Julia FERRONATO(1)

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Abstract/Resumen: Despite chemotherapy remaining as a primary therapeutic option for aggressive breast cancer (BC), its effectiveness is limited by intrinsic or acquired resistance and associated adverse effects. Therefore, new therapeutic strategies are needed. Previously, we demonstrated that the calcitriol analogue EM1 decreases the viability, migration and invasion of the 4T1 triple-negative BC (TNBC) cells. Additionally, we reported that UVB1, another calcitriol analogue synthetized by our group, reduces the viability of cells derived from the TNBC - Patient-Derived Xenografts (PDX). Hence, the aim of the present study was to continue evaluating the antitumoral effects of the calcitriol analogues EM1 and UVB1 on aggressive BC cells, alone or in combination with low concentrations of Paclitaxel (PTX). We found a synergistic effect by combining EM1 or UVB1 with non-effective PTX concentrations on viability of 4T1 cells. The resulting Combination Index values of Chou & Talalay method were 0.80059 and 0.13491 for EM1-PTX and UVB1-PTX combinations, respectively. In addition of our previous result on 4T1 cell migration, EM1 displayed antimigratory effects on MDA-MB-231 TNBC cell line ($p<0.001$). In contrast, UVB1 had no effect on these cells. However, interestingly, the combination of the analogues with non-effective concentrations of PTX over 4T1 cell migration displayed a better effect than drugs alone (EM1-PTX: $p<0.05$; UVB1-PTX: $p<0.001$). Finally, a pilot in vivo assay was conducted to test the sensitivity of the TNBC-PDX410 to UVB1. A reduction in in vivo tumor volume was detected after 18 days of UVB1 treatment at 40 µg/kg of body weight administrated three times a week ($p<0.05$). Altogether, these results suggest the potential use of these vitamin D analogues as antitumor agents, alone or as a complement to conventional chemotherapy.

0408 - ANTITUMORAL EFFECTS OF PLEUROTUS OSTREATUS I-FRACTION IN BREAST CANCER

Rocío RAMBURGER (1) | María Julia FERRONATO(1) | Josefina Alejandra GUEVARA(1) | Juan Manuel CUESTAS(2) | Pablo Daniel POSTEMSKY(2) | Alejandro Carlos CURINO(1) | María Marta FACCHINETTI(1) | Eliana Noelia ALONSO(1)

INIBIBB-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (1); CERZOS-CONICET, DEPTO. BIOLOGÍA, BIOQUÍMICA Y FARMACIA-UNS (2)

Abstract/Resumen: Mushrooms are considered as "small pharmaceutical factories" producing hundreds of bioactive compounds, many of which have shown to exert antitumoral activity in different types of cancer. Argentina has a high mushrooms diversity and important scientific-technological development applied to its cultivation. However, the antitumoral phytotherapeutic potential of edible mushrooms cultivated in our country has not yet been considered. In this context, the purpose of the current study is to determine the antitumoral activity in breast cancer of Pleurotus ostreatus I-Fraction, an extract of water-soluble polysaccharides obtained from fruiting body, initially evaluating its potential immuno-independent antitumoral activity. To achieve the proposed objective, we employed a murine mammary adenocarcinoma 4T1 cells line and performed cell viability assays by colorimetric assay with crystal violet, cell cycle analysis by flow cytometry, and wound healing assay. We found that P. ostreatus I-Fraction at concentration from 2.5 mg/mL and ranging from 1.0 to 2.5 mg/mL decreased the viability of 4T1 cells in a concentration-dependent manner, at 24 hours and 48 hours respectively ($p<0.001$). These results also demonstrate a time-dependent effect of I-Fraction on 4T1 cells viability. In addition, P. ostreatus I-Fraction (2.5 mg/mL, 48 h) increased the number of 4T1 cells in the subG0/G1 phase (I-Fraction= 9.05 vs. vehicle= 2.3 %, $p<0.001$) and decreased those in the G0/G1 phase, compared to vehicle (I-Fraction= 42.3 vs. vehicle= 48.77 %, $p<0.001$). These results suggest that I-Fraction decreases 4T1 cell viability through an induction in cell death, without affecting cell cycle progression. By another hand, we found that I-Fraction decreased migratory capability of 4T1 cells at 13 h of treatment, compared to vehicle ($p<0.01$). In conclusion, these results demonstrate the antitumor activity of Pleurotus ostreatus I-Fraction on breast cancer cells.

0409 - P300 INVOLVEMENT IN METASTATIC PROCESS OF TRIPLE NEGATIVE BREAST CANCER

Guillermina Ana GALLARDO | Valentina CLEMENTE | Marilina MASCARÓ | María Marta FACCHINETTI | Alejandro Carlos CURINO | María Eugenia FERMENTO

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Abstract/Resumen: Triple negative breast cancer (TNBC) are a heterogeneous group of tumors which lack specific molecular targets. Therefore, it is necessary to investigate potential tumor markers for this subtype of BC. Recent studies indicate that p300 has a pro-metastatic role in BC and we have previously shown that inhibition of p300 decreases cellular migration and invasion in a TNBC cell line. Therefore, in this work we aimed to analyze the expression and localization of p300 and its association with markers of tumor progression and clinic-pathological parameters in human TNBC. Also, we investigated the molecular mechanisms through which p300 inhibition impaired the processes previously mentioned. In TNBC biopsies (n= 45), we found that higher levels of cytoplasmic p300 correlates with lower tumor stages and a better overall patient survival (IHC, $p<0.05$). In TNBC (MDA-MB-231) and hormone-independent BC (LM3) cell lines, the genetic silencing of p300 induced an increase in the levels of