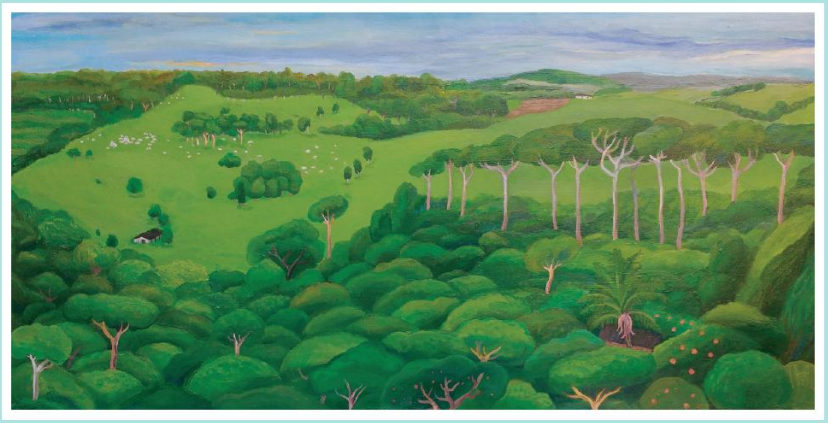
2018

medicina

BUENOS AIRES VOL. 78 Supl. III - 2018



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for the influx of CD11b+ cells, Ly6G+ myeloid cells and Siglec-F+ eosinophils into the LP of infected mice. Shiga toxin may not induce a differential innate immune response in the gut, at least on the leukocyte cell subsets studied in the present work.

227. (654) **PARAPROBIOTICS AS IMMUNOPOTENTIATING AGENTS OF MYELOPOIESIS**

Gutiérrez Florencia[1], Andrés Daniel Gramajo Lopez[1], Jorge Armando Jimenez Avalos[1], Héctor Maximiliano Ivir[2], Julio Cesar Villena[1], Susana Alvarez[1,2], Susana Salva[1]
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Myelosuppression is the major dose-limiting toxicity of systemic cancer chemotherapy. At present, hematological rescue techniques are applied to reduce the chemotherapy-induced neutropenia that includes new adverse effects. Probiotic acid lactic bacteria (BL) has shown to be promising safe agents to reduce myelosuppression. We demonstrated that the dietary supplementation with probiotic *Lactobacillus rhamnosus* CRL 1505 (Lr05) improved steady-state and emergency granulopoiesis, the respiratory innate immune response and the resistance against respiratory pathogens in immunosupressed hosts. While the viability of the BL is an important factor to achieve optimal protective effects, it is also possible to stimulate immunity using non-viable BL. The aim of this work is to study the ability of paraprobiotics (cell fractions) from Lr05 to minimize myelosuppressive effects derived from chemotherapy. Adult Swiss-mice were orally treated with paraprobiotis, peptidoglycan (PG group) and cellular wall (CW group), during 15 consecutive days (8 μg/mice/day). On day 6, paraprobiotic-treated and untreated control mice received one intraperitoneal dose of cyclophosphamide (Cy) (150 mg/kg). Before Cy-injection, both PG and CW groups increased the peroxidase (Px) score in blood and bone marrow (BM) myeloid cells compared with control ($p<0.05$). Cy impaired steady-state myelopoiesis However, the paraprobiotic treatments were able to significantly increase BM hematopoietic stem cells ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$), myeloid multipotent precursors (Lin^+CD34^+), myeloid cells ($Gr\text{-}1^+Ly6G^+Ly6C^-$) and Px+ cells with respect to the control group ($p<0.05$). Besides, the CW treatment was more effective than the PG to allow an early recovery of these parameters. This, in turn, led to an early increase blood neutrophils and Px score. In conclusion, both PG and CW obtain from *L. rhamnosus* CRL1505 were able to improve BM steady-state myelopiesis in mice undergoing chemotherapy

ONCOLOGÍA / ONCOLOGY ORAL SESSION 1

228. (86) **MUSCARINIC RECEPTORS EXPRESSED IN NON-TUMORIGENIC MCF-10A CELLS INDUCED A MALIGNANT PHENOTYPE THAT ACTIVATES HUMAN ENDOTHELIAL CELLS**

Martinez Paola[1], Maria Gabriela Lombardi[1], Francisco Sanchez[1], Gabriel Fiszman, Maria Elena Sales[1]
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Our laboratory reported the participation of muscarinic acetylcholine receptors (mAChR) in different steps of tumor progression in murine and human breast cancer. To confirm the contribution of mAChR to malignant transformation, we developed a new cell line by stably transfecting the non-tumorigenic human mammary cell line MCF-10A with mAChR, subtypes 3 and/or 4. Transfected cells acquired the ability to generate three-dimensional structures (spheroids) that mimic the first stages of tumor growth in vivo. Tumor microenvironment has a crucial role in tumorigenesis, particularly endothelial cells, as they form the tumor microvasculature. Considering the later, we analyzed the effect of spheroid-derived conditioned media (S-DCM) from different days of culture on human endothelial cells-1 (HMEC-1) viability, vascular endothelial growth factor-A (VEGF-A) expression and tubulogenesis. S-DCM from 14 days significantly increased HMEC-1 cell viability (mAChR3:153.8%+11,7; mAChR4: 211.2+13.3%; mAChR3/4:171.9+13.5%; n=3) in comparison with conditioned media from MCF-10A (100+18.6%), and in a similar manner than MCF-7 tumor cells (155.6+16.7%; n=3). VEGF-A expression was up-regulated when HMEC-1 cells were treated with S-DCM of 14 days (mAChR3:167.4+5.6%; mAChR4:61.5+10.7%; mAChR3R4:126.7+7.7%; n=3) comparably to S-DCM from MCF-7 tumor cell spheroids (109.5+23.0; n=3). Finally, we investigate the effect of S-DCM of 14 days on HMEC-1 cells in a tube formation assay (mAChR3:119.4+9.9; mAChR4: 117.5+9.8; mAChR3R4:102.2+7.2; MCF-7:130.6+6.33) vs. HMEC-1 cells without treatment. In conclusion, the transfection of non-tumorigenic breast cells MCF-10A with mAChR induces a malignant phenotype that triggers three-dimensional growth and the liberation of pro-angiogenic mediators stimulating HMEC-1 cell viability, VEGF-A expression and tubulogenesis in a similar manner to that observed in breast tumors.

229. (139) **RSUME INHIBITS TYPE 2 VHL MUTANTS FUNCTION LEADING TO TUMORAL ANGIOGENESIS BY INHIBITING THE ASSEMBLY OF THE VHL FUNCTIONAL COMPLEX**

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von Hippel-Lindau (VHL) disease is associated with the development of high vascularized tumors due to Hypoxia Inducible Factors (HIF) deregulation caused by mutations in VHL gene. VHL is the substrate recognition component of the E3 ligase complex, composed of Cullin2, Elongin B, Elongin C, and pVHL, that participates in the oxygen-sensing system that drives HIF degradation.Certain mutations retain a partial function on HIF downregulation implying additional mechanisms involved in VHL mutants loss of function. We have already demonstrated that RSUME interacts with VHL, and inhibits its function, leading to HIFs-α stabilization. Even more, we also found the same action of RSUME on representative Type 2 mutants (VHLY112H; VHLR167Q; VHLL188V) and we found this mechanism was independent of VHL sumoylation status.The aim of this work is to reveal the molecular mechanism behind RSUME potentiation of Type 2 VHL phenotype and its functional impact. COS-7 cells were cotransfected with Flag-VHLY112H-GFP or Flag-VHLR167Q-GFP or Flag-VHLL188V-GFP, the ECV complex components (Cullin2, Elongin B, Elongin C) and/or V5-RSUME. By VHL inmunoprecipitation we observed that RSUME interaction with VHL type 2 mutants impairs the ECV complex assembly, which inhibits its function.
In RCC-786-O clones expressing VHL, VHLK171R, VHLL188V or VHLL188V/K171R, co-expression of shRNA against RSUME resulted in a decrease of VEGF mRNA. EA.hy926 endothelial cells cultured in conditioned media of these clones, in which RSUME was silenced, decrease the capillary-like structures formation. Mice injected with these stable clones presented new vessels around the injection area, but those clones in which RSUME expression was knocked-down showed a decrease in vessel density. This confirms that in absence of RSUME, VHL Type 2 mutant become more potent and might limit early tumoral angiogenesis.RSUME is critical in VHL mutants deregulation that leads to VHL disease onset.Supported by ANPCyT, CONICET, UBA and FOCEM (COF 03/11).

230. (195) **DETECTION OF CIRCULATING MIRNAS AS POSSIBLE BIOMARKERS OF PROSTATE CANCER ASSOCIATED TO METABOLIC SYNDROME**

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Prostate cancer (PCa) is the most common type of cancer and is the third cause of death by cancer among males in Argentina. Metabolic syndrome (MeS) is a cluster of pathophysiological disorders whose diagnose requires the detection of, at least, three of the following factors: visceral adiposity, high triglycerides, low High Density Lipoprotein (HDL) cholesterol levels, high blood pressure and elevated fasting glucose levels. A recent meta-analysis found a significant

correlation associating MeS with more aggressive PCa tumors and biochemical recurrence. C-terminal binding protein (CTBP1) is a transcriptional co-repressor of many tumor suppressor genes. Binding either NAD+ or NADH is necessary for CTBP1 activation; however, CTBP1 affinity is 100-fold higher for NADH making it a molecular sensor of the metabolic state of the cell and an interesting link between PCa and MeS. Recent years have seen an overflow of reports regarding miRNAs role in cancer. Many reviews have been published on miRNAs deregulation in cancer, both as cause and consequence, and as possible biomarkers or therapeutic molecules. In this work our aim was the identification of circulating miRNAs to be used in the near future as biomarkers of PCa associated to MeS. To this end, we analyzed serum samples collected from mice bearing xenotransplants and detected 4 miRNAs by RT-qPCR. Among them miR-30b-5p was significantly down regulated in the circulation of MeS mice that were inoculated with control CTBP1 expression cells compared to the mice inoculated with CTBP1 depleted cells. We also analyzed circulating miRNA levels on PCa patient serum samples that were clustered depending on Gleason Score and parameters associated to MeS. In addition, we analyzed serum samples from benign prostatic hyperplasia (BPH) patients and healthy donors, which were clustered according to MeS parameters. We identified many candidates for further analysis as possible biomarkers of PCa associated to MeS.

231. (271) **THE HUMAN ADIPOSE TISSUE SECRETE COMPONENTS THAT REGULATE THE TUMOR AND NON TUMOR RENAL EPITHELIAL CELLS BEHAVIOR**

Flavia Bruna[1, 4], Leonardo R Romeo[2], Silvina E Gómez[1], Flavia E Santiano[1], Corina V Sasso[1], Leila E Zyla[1], Constanza Lopez-Fontana[1], Rubén W Carón[1], Virginia Pistone Creydt[1, 3]
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An essential information exchange is established between renal epithelial cells and adipose/fibroblastic stroma. In the present work, we evaluated the conditioned media (CMs) effects of human adipose tissue explants from normal (hRAN) and tumor (hRAT) kidney on: proliferation, adhesion and migration of tumor (786-O, ACHN, Caki-1) and non tumor (HK-2) renal epithelial cells. We also evaluated pERK/ERK y pPI3K/PI3K changes in different cell lines incubated by 2 and 24 h with hRAN-, hRAT, or control-CMs.Human renal adipose tissues were obtained from patients with renal cell carcinoma (hRAT, n=9) and kidney donors (hRAN, n=10). The CMs of hRAN and hRAT were collected 24 h post incubation and cells were treated with CMs. Proliferation (MTT assay), adhesion and migration (wound-healing and transwells assay) were evaluated in 786-O, ACHN, Caki-1 and HK-2 cell lines incubated with different CMs. The expression of pERK/ERK and pPI3K/PI3K on cell lines incubated with CMs (WB assay). Statistical differences among experimental conditions were evaluated by one-way ANOVA with Tukey´s post hoc tests. All cell lines showed a significant decrease in cell adhesion ($p<0.05$) and increase in cell migration ($p<0.05$) after incubation with hRAT-CMs vs. hRAN- or control-CMs. Surprisingly, HK-2, 786-O and ACHN cells showed a significant decrease in cell migration ($p<0.05$) after incubation with hRAN-CMs vs. control-CMs. The expression of pERK/ERK was found decreased ($p<0.05$), and pPI3K/PI3K increased ($p<0.05$), in HK-2 and ACHN incubated with hRAT-CMs vs. hRAN- or control-CMs ($p<0.05$). No differences on proliferation of cell lines were found after 24 or 48 h of treatment with CMs.In conclusion, the adipose microenvironment could be regulating the behavior of tumor and non tumor human renal epithelial cells. The tumor stroma should be taken into consideration when dealing with a malignancy.

232. (517) **EPIGENETIC INHIBITORS ELIMINATE MELANOMA BRAF V600E CELLS THAT PERSIST AFTER BRAF INHIBITION**

Florencia Madorsky Rowdo, Antonela Baron, María Marcela Barrio, Jose Mordoh[1]
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Approximately one-half of melanoma patients harbor the BRAFV600 driver mutation, the most common being BRAFV600E, which leads to the activation of MAPK proliferative and survival pathway. BRAF inhibitors are extensively used to treat BRAF-mutated metastatic melanoma but unfortunately acquired resistance occurs in the majority of patients. Resistance mechanisms involve mutations or changes in gene expression that result in the reactivation of MAPK signaling or activation of other proliferative and survival pathways. We studied the effect of PLX4032 (BRAFV600 inhibitor) long-term treatment on sensitive V600E BRAF-mutated melanoma cell lines. After several weeks of long-term in vitro treatment with PLX4032 the majority of the melanoma cells died whereas some remained viable and quiescent. We named this population SUR cells. Discontinuing treatment of SUR cells with MAPK inhibitors allowed the population to regrow and these cells retained drug sensitivity equal to that of parental cells. We performed RNA-seq in order to determine differences between parental cells and this persistent quiescent population, finding that SUR cells presented changes in the expression of 1509 genes ($p<0.05$). These results suggest that the SUR phenotype may be determined by epigenetic changes. We found that SUR cells changed the expression of epigenetic enzymes. We analyzed the sensitivity to different epigenetic inhibitors and we found that both parental and SUR cells were sensitive to the HDAC (SAHA and mocetinostat) and CDK9 (CDKI-73) inhibitors. These epigenetic inhibitors induced apoptosis and reduced proliferation and invasion in a 3D model both in the parental and SUR populations. We propose the combination of PLX4032 with epigenetic inhibitors in order to achieve a complete elimination of SUR cells that persist after BRAF inhibitor treatment.

233. (79) **ANTI-TUMOR ACTIONS OF CYTOTOXIC DRUGS PLUS MUSCARINIC AGONISTS ON HUMAN TRIPLE NEGATIVE BREAST CANCER CELLS**

Agustina Salem[1], Yamila Sanchez[1], Alejandro Español[1], María Elena Sales[1]
[1]*CEFYBO-CONICET-UBA*

The administration of low doses of cytotoxic drugs alone or combined with repurposing drugs scheduled with short inter-dose intervals is called metronomic therapy (MT). MT is a new strategy in cancer treatment, since it exhibits high effectiveness and low incidence of side effects. We previously demonstrated that the activation of muscarinic receptors (M) can modulate breast cancer cell viability. Triple negative (TN) breast tumors are highly aggressive, and the effectiveness of pharmacological treatment is low probably due to the absence of a specific target. Here, we analyzed the effect of a combination of subthreshold concentrations of a muscarinic agonist, carbachol (CARB) or arecaidine propargyl ester (APE) (non-selective or selective for M2 subtype respectively) with paclitaxel (PX) or doxorubicin (DX), two cytotoxic drugs used in breast cancer treatment, on MDA-MB231 or MDA-MB468 TN tumor-derived cell lines. By MTT assay we observed on MDA-MB231 cells that the combination of PX+CARB or PX+APE reduced cell viability (23.9±2.5%; 23.5±7.1% respect to control; $p<0.05$). When we combined these muscarinic agonists with another cytotoxic drug, DX, we also observed a reduction in cell viability (DX+CARB: 27.4±4.2%; DX+APE: 30.7±1.6%; $p<0.001$ vs. control). To confirm these results we analyzed the effect of these drugs on another TN cell line, MDA-MB468, obtaining similar results (PX+CARB: 50.3±2.4%; PX+APE: 26.9±3.6%; $p<0.001$ vs. control). When we combined CARB or APE with DX we observed the same effect (DX+CARB: 21.1±0.7%; DX+APE: 31.2±0.9%; $p<0.05$ vs. control). None of the combined treatments had effect on the non tumorigenic cell line, MCF-10A. These results suggest that the combination of low doses of cytotoxic drugs with muscarinic agonists can reduce cell viability and could be used as a new strategy to treat TN breast tumors in humans.

REPRODUCCIÓN / REPRODUCTION ORAL SESSION

234. (152) **POSSIBLE EFFECT OF ROUNDUP ON SERTOLI CELL FUNCTION**

Agostina Gorga[1], Gustavo Rindone[1], Cristian Sobarzo, María del Carmen Camberos[1], Eliana Pellizzari[1], María Fernanda