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# REUNIÓN CONJUNTA DE SOCIEDADES DE BIOCIENCIAS

## LXII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

## LIII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN BIOQUÍMICA Y BIOLOGÍA MOLECULAR (SAIB)

## LXV REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

## REUNIÓN DE LA SOCIEDAD ARGENTINA DE ANDROLOGÍA (SAA)

## XLVI REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE BIOFÍSICA (SAB)

## XIX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE BIOLOGÍA (SAB)

## XLIX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE FARMACOLOGÍA EXPERIMENTAL (SAFE)

## REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE FISIOLOGÍA (SAFIS)

## REUNIÓN DE LA SOCIEDAD ARGENTINA DE HEMATOLOGÍA (SAH)

## XXIX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE PROTOZOOLOGÍA (SAP)

13-17 de noviembre de 2017
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The ability of cells to adhere and simultaneously probe their mechanical environment is central to many developmental, homeostatic but also pathological processes. Yet, the molecular mechanisms that govern mechanotransduction during cell adhesion and invasion are complex and remain incompletely understood. We determined the importance of studying integrins in genetically modified cell models that only express either family of fibronectin (FN)-binding integrins. Using biochemical assays in combination with mass spectroscopy, traction force microscopy and micropatterns, we observed that α5β1-integrins (pKO-β1) promote the formation of small nascent adhesions with high turnover, low RhoA activation and high force, while αVβ3 (pKO-αV) promotes adhesion maturation leading to large focal adhesions connected to contractile stress fibers resulting in high RhoA and low force, while cells expressing both integrins contain small and large adhesion structures and intermediated GTPases activites. Therefore, we observed that the levels of integrins expression and subtype in different cells lines affect G-actin polymerization, MRTF-A/SRF activation and the ubiquitin-like modifier interferon-stimulated gene 15 (ISG15) expression that promotes cell migration and invasion. Interestingly, the malignant breast cancer cell line MDA-MB-231 expressing high levels of β1 integrins and the β1-class expressing pKO-β1 cells showed high ISG15 expression and high amounts of ISGylated proteins, which we could show are directly responsible for cell invasion. In contrast, the non-invasive tumor cell lines MDA-MB-468 or MCF-7 and the αV-class integrin expressing pKO-αV cells, which display low levels of β1 integrins, ISG15 and ISGylated proteins failed to invade a 3D matrix. The future findings have important implications for our understanding of cancer progression and will help identifying new targets for future therapies.

Keywords: Breast Cancer, Integrins, Rho-GTPases, mechanosignaling

(1138) **ANTI-TUMORAL PROPERTIES OF CHLOROGENIC ACID: MAIN BIOACTIVE COMPONENT OF YERBA MATE**

Rocío García Lazaro, Norailys Lorenzo, Lorena G. Caligiuri, Humberto Lamdan, Daniel F. Alonso, Hernán G. Farina
*Laboratorio de Oncología Molecular. Departamento de Ciencia y Tecnología. Universidad Nacional de Quilmes*

*Abstract:* Yerba Mate (*Ilex paraguariensis*) is a native South American tree but Yerba Mate tea is growing in popularity around the world by biological properties. This plant contains several active phytochemicals which are responsible for its health benefits. One of the most abundant polyphenol compounds in Yerba Mate is chlorogenic acid. It has been reported many biological properties for this polyphenol, including antioxidant, anti-inflammatory, antiviral, and anticancer activities, and may be responsible for the reduced risk of some chronic disease. We previously showed the anti-tumoral properties of Yerba Mate extract in several experimental systems. The aim of this study was to explore whether chlorogenic acid is the molecule responsible of biological effects observed in Yerba Mate extract. Total polyphenol concentration was measured using Folin-Ciocalteau method, with a mean of 0.03 gallic acid equivalents/mg Yerba Mate. Several classes of chemical constituents as caffeoyl derivatives (chlorogenic acid and caffeic acid) and flavonoids (rutin, kempherol and quercetin) were quantified by HPLC. *In vitro* assays were performed using tumor cell lines from different localization (CT26, MDA-MB-231, H125, Colo205, SN12C, PC3). Both, Yerba Mate extract and chlorogenic acid inhibited cell proliferation in a dose-dependent manner. However, Yerba Mate extract was the most potent inhibitor of cell proliferation at a concentration lower than the concentrations used of chlorogenic acid (ranged $IC_{50}$= 0.027-0.1 mM for YM and 0.312-0.75 mM for chlorogenic acid). On the other hand, we demonstrated that both Yerba Mate extract and chlorogenic acid modulate cell adhesion, migration and the invasive capacity of tumor cells. Our results suggest that the *in vitro* biological effects of Yerba Mate extract are not only due to chlorogenic acid but also could be additive and synergistic effects among the different bioactive components present in Yerba Mate.

Keywords: chlorogenic acid, Yerba Mate, tumor cell, phytochemical, antioxidant

(650) **COMPARATIVE PROTEOMICS OF SOLUBLE FACTORS SECRETED BY HUMAN BREAST ADIPOSE TISSUE FROM TUMOR AND NORMAL BREASTS**

Sabrina Johanna Fletcher (1), María Belén Hapon (2), María Paula Marks (1), Anabela Ursino (3), Natalia Santiso (3), María Luján Crosbie (3), Alicia Rita Amato (3), Alberto Gutiérrez (3), Rubén Dreszman (4), Eduardo Callegari (5), Judith Toneatto (1), Luciano Vellón (1), Rubén Walter Carón (2), Juan Carlos Calvo (1), Virginia Pistone Creydt (2)
*(1) IByME – CONICET. (2) IMBECU – CONICET. (3) Complejo Médico Policial Churruca-Visca. (4) Clínica de Microcirugía. (5) University of South Dakota Sanford School of Medicine, Vermillion, SD USA.*

Epithelial-stromal cell interaction is a crucial factor in cancer progression. Adipose tissue is the main stromal component of breasts. We have previously demonstrated that conditioned media (CMs) from human adipose tissue explants of tumor breasts (hATT) differentially regulate proliferation, adhesion and migration of breast cancer epithelial cells compared with CMs from human adipose tissue explants of normal breasts (hATN). Now, we intend to identify the proteins present in those CMs. For this, we separated in polyacrylamide gels proteins in CMs –hATT (n=6) and –hATN (n=3). Aliquots from these CMs were analyzed by means of 2D nano-LC-MS/MS (mass spectrometry). The data was analyzed using ProteoIQ (Premier Biosoft) and FunRich softwares. In addition, CMs –hATT (n=6) and -hATN (n=5) were assayed using a 42 Cytokine Antibody Array. We found that CMs-hATT present more protein diversity than CMs-hATN. Moreover, CMs-hATT expressed greater amount of proteins involved in biological processes such as signal transduction and cell communication; energy metabolism; cell growth; and immune response. Specifically, levels of apolipoprotein AI and AII (lipid metabolic processes), C3 complement factor (immune system) and vimentin and desmin (mesenchymal cells glycoprotein related to an invasive breast cancer phenotype) were significantly increased in CMs-hATT vs. CMs-hATN (cut-off 5-fold change). Furthermore, a multivariate discriminant analysis of the cytokines detected by the array showed that IL-6, MCP-2 and GRO cytokines are sufficient and necessary to differentiate CMs-hATT from CMs-hATN. In addition, this analysis showed that the levels of these three cytokines taken together correlate with tumor stage of CMs-hATT and with BMI of CMs-hATN. These results allowed us to identify proteins potentially responsible for the observed effects, and let us proposed stromal IL-6, MCP-2 and GRO as potential markers of the stage of the disease.

*Keywords:* breast cancer, adipose tissue, epithelial-stromal interaction, proteomics analysis.

(1547) **DIFFERENTIAL ANTITUMORAL EFFECTS BETWEEN THE ANALOGUES OF CALCITRIOL SG1 AND EM1.**

Silvina Mariela Grioli (1,2), María Julia Ferronato (1), Diego Javier Obiol (1), Josefina Alejandra Guevara (1), Mario Alfredo Quevedo (3), Gabriel Radivoy (2), Cristian Vitale (2), Alejandro Carlos Curino (1), María Maria Faccihinetti (1)
*(1) Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), Universidad Nacional del Sur (UNS) CONICET, Departamento de Biología, Bioquímica y Farmacia (UNS), Bahía Blanca, Argentina. (2) Laboratorio de Química Orgánica, Instituto de Química del Sur (INQUISUR), Universidad Nacional del Sur (UNS) CONICET, Departamento de Química (UNS), Bahía Blanca, Argentina. (3) Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA-CONICET), Facultad de Ciencias Químicas, Ciudad Universitaria, Universidad Nacional de Córdoba, Córdoba, Argentina.*

1α,25-dihydroxyvitamin $D_3$ (calcitriol) shows potent growth-inhibitory properties on different cancer cell lines although its hypercalcemic effects have severely hampered its therapeutic application. Therefore, it is important to develop synthetic analogues that retain or even increase the antitumoral effects without causing hypercalcemia. Based on the previous evidence of the potent antitumor effects

of the synthetic alkynylphosphonate analogue EM1, we have now synthesized a novel analogue called SG1, which bears a vinylphosphonate in its side chain. The aim of the present work was to evaluate the calcemic activity in mice and the antitumor effect of SG1 on different cancer cell lines, comparing them with that exerted by calcitriol and by EM1. In addition, we performed computational modeling studies in order to analyze and compare the affinity of the compounds to the vitamin D receptor (VDR). By manual cell count we observed that SG1 exerted a slight decrease in the viability of the HCT116 ($IC_{50}$: 3,13 nM; $p< 0.05$) and LM3 ($IC_{50}$: 0,19 nM; $p< 0.001$) cell lines whereas it did not affect the viability of HN12, T47D, U251 and T98G cells. By wound healing assays, we observed reductions in the migration rates of the LM3 ($p< 0.001$) and T98G ($p< 0.05$) cell lines, whereas it did not affect the migration of the HCT116, U251, GL26, HN12, T47D. Calcemic assays performed in CF1 mice showed that, similarly to EM1, the new analogue SG1 did not cause hypercalcemia (at 5 $\mu$g/kg) or toxic effects. Computational studies were performed using as reference the crystallographic structure of the calcitriol-VDR complex (PDB code: 1DB1) and conclude that SG1 binds with lower affinity to VDR than the other two compounds. In conclusion, these results suggest that the modifications in the lateral side chain of analogue SG1 (vinylphosphonate instead of alkynylphosphonate) affect VDR binding affinity and the antitumoral effects previously observed for EM1, while not changing the calcemic activity.

*Keywords*: Analogues, Antitumor agents, Calcitriol, Cancer, Cell lines.

(553) **DUAL APOTOTIC/NECROTIC RESPONSE INDUCED BY PHOTODYNAMIC TREATMENT WITH A Zn(II) CATIONIC PHTHALOCYANINE IN MELANOMA CELLS**

Federico Valli, María Cecilia García Vior, Leonor Roguin, Julieta Marino
*IQUIFIB, UBA-CONICET*

Melanoma is an aggressive form of skin carcinoma, highly resistant to traditional therapies. Photodynamic therapy (PDT) is an alternative form of treatment, which combines a photosensitizer, visible light and molecular oxygen to produce reactive oxygen species (ROS) that selectively destroy target cells.

In order to find an efficient photosensitizer to be used in melanoma treatment, we evaluated the effect of a sulfur-linked cationic zinc(II) phthalocyanine (Pc13) on a panel of melanoma cells (B16F0, B16F10, WM35, M1/15, A375). Incubation with Pc13 and irradiation, diminished cell viability in a concentration and light dose-dependent manner in all the cell lines studied, with $IC_{50}$ values ranging from 0.20±0.03 μM to 3.60±0.23 μM for B16F0 and A375 cells, respectively. The most sensitive melanoma cells B16F0 were further employed for studying the mechanisms of cell death triggered by Pc13. Acridine orange/ethidium bromide dual staining showed morphological changes characteristic of both necrosis (42±4%) and apoptosis (21±2%) 3h post irradiation of cells treated with 0.2 $\mu$M Pc13. Under these experimental conditions, a significant decrease in the levels of Bcl-2, Bcl-xL and Bid, and a reduction of pro-caspase-3 were observed by Western Blot, evidencing an apoptotic response. Furthermore, a time dependent increase of hypodiploid cell population and cell cycle arrest in G0/G1 were assessed by propidium iodide staining and flow cytometry analysis. In addition, permeabilization of plasma membrane, as sign of necrosis, was evaluated by measuring the release of lactate dehydrogenase (LDH). A light dose and photosensitizer concentration dependent increase of LDH activity was detected in Pc13 treated-cells culture mediums.

Taken together, these results indicate that a dual apoptotic and necrotic response is triggered by Pc13 photoactivation in melanoma cells, suggesting that combined mechanisms of cell death could result in a promising alternative for melanoma treatment.

*Keywords:* photodynamic therapy, melanoma, phthalocyanine, antitumoral action

(1701) **IMIQUIMOD-INDUCED INHIBITION OF ANTIOXIDANT ENZYMES AND REACTIVE OXYGEN SPECIES ACCUMULATION IN MURINE HEMANGIOMA CELLS.**

Rodrigo Rocco, Hernán Reingruber, Rosa Wainstok, Adriana Cochón, Silvina Gazzaniga
*Universidad de Buenos Aires, Fac de Ciencias Exactas y Naturales, Dpto. de Química Biológica. IQUIBICEN- Universidad de Buenos Aires. Instituto de Química Biológica de la Fac.de Ciencias Exactas y Naturales.*

Infantile hemangiomas (IH) are the most common benign tumours of infancy, however intervention may be required when major complications are developed. Imiquimod (IQ), a TLR7/8 agonist, is a therapeutic alternative and previous *in vitro* studies of our laboratory have shown a cytotoxic selective effect of IQ towards hemangioma cells in terms of viability, migration and apoptosis triggering. The aim of this study was to investigate the ability of IQ to trigger reactive oxygen species (ROS) generation and its influence on antioxidant enzymes, prior to apoptosis of murine hemangioma cells. H5V cell line was treated with IQ (0, 5, 10 and 50 μg/mL) for 2, 4 or 12 hours and analyzed for ROS generation and mitochondrial stability by flow cytometry using fluorescent probes DCF-DA and MitoTracker Red CMXRos, respectively. Antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) activities were assessed through disappearance of $H_2O_2$ (μmol $H_2O_2$/min*mg protein) and inhibition of the epinephrine oxidation (USOD/mg protein) respectively. Early after treatment with IQ (2 hs) there was a steep drop (70±12%; $p<0.05$) in the specific activity of CAT, accompanied by a 40%-increase in ROS levels ($p<0.05$). When treating H5V cells for 4 hs, inhibition of the activity was about 40% for both CAT and SOD. ROS increased from 40 to 100% for 5-50 μg/mL ($p<0.05$) along with (50±15)% loss of mitochondrial membrane potential. After 12 hs treatment, there was a restoration of CAT activity at IQ concentrations ≤ 10 μg/mL and induction at 50 μg/mL. SOD showed a 25% increased activity and mitochondrial stability remained impaired (40±10)% for ≥ 5 μg/mL IQ. In conclusion, IQ treatment of H5V cells would induce ROS accumulation, mitochondrial dysregulation and inhibition of antioxidant enzymes. These early modifications of the oxidative status may contribute with previously reported IQ-induced apoptosis in H5V cells.

**Keywords:** imiquimod, hemangioma, ROS, antioxidant enzymes.

(1325) **N-TERMINAL PORTION OF C-FOS AS A NEW THERAPUETIC STRATEGY FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME TUMORS**

César Germán Prucca, Ana Cristina Racca, Fabiola Noelia Velazquez, Beatriz Leonor Caputto
*CIQUIBIC-UNC*

The survival time for patients with Glioblastoma multiforme has not improved significantly over the last ten years with an average survival period for these patients of ~1 year after diagnosis, thus representing the most aggressive and lethal type of tumors of the central nervous system (CNS). We found that in addition to its role as an AP1 transcription factor, c-Fos activates the rate of synthesis of phospholipids, key components for membrane biogenesis, at the endoplasmic reticulum. Furthermore, we determined that the regulation of this metabolism is implicated in tumor biology, sustaining the exacerbated growth characteristic of brain tumor cells. We also found c-Fos overexpressed in brain tumors co-localizing with components of the endoplasmic reticulum contrasting with the lack of detectable expression of c-Fos in normal CNS. These results point to c-Fos as a potential new target for glioblastoma treatment. Consequently, the aim of the present work was to test N-terminal deletion mutants of c-Fos as possible negative dominants of the lipid synthesis activation capacity of c-Fos. Using several *in vitro* approaches, (transfection, profection of recombinant proteins and generation of stable cell lines) we identified negative dominants whose overexpression inhibits proliferation of T98G cells, we evaluate the induction of cell death and we dissect the domains of N-terminal portion of c-Fos (NA) involved in the physical interaction of c–Fos with enzymes that it activates such as phosphatidylinositol 4 kinase IIα (PI4KIIα) using FRET microscopy. Moreover, the negative dominance of NA resulted effective in an *in vivo* model of CNS tumors using T98G xenografts on immunodeficient NOD-SCID mice. Taken together, our results point to specific domains of NA as possible new therapeutic strategies for the treatment of glioblastoma multiforme.