ployed a syngeneic model of C57BL/6 mice bearing subcutaneous B16F0 melanoma tumors. First, Pc13 biodistribution was examined after intratumoral administration using an *in vivo* imaging system. We found that maximum retention of the photosensitizer in the tumor was reached at 3 h post administration. Moreover, the presence of Pc13 in liver, intestines and kidneys suggested possible elimination pathways of this phthalocyanine. Laser irradiation (250 J/cm^2) after 3 h of intratumoral injection of 2 mg/kg Pc13 significantly reduced tumor volume at the end of the experiment (65%, $p<0.01$), compared to control groups. Body weight and histological characteristics of different tissues stained with hematoxylin-eosin were not altered in treated-mice, indicating non-toxicity of Pc13-PDT. Histological analyses of tumor sections showed a marked increase of necrotic areas (57% vs. 5%) and a reduction of PCNA staining after treatment. Furthermore, increased levels of Bax, active caspase 3 and diminished expression of Bcl-2 were observed in treated-tumors by Western Blot and immunofluorescence assays, indicating apoptotic cell death. In addition, higher levels of LC3-II and Beclin-1 demonstrated the participation of an autophagic response after Pc13-PDT. In conclusion, our results showed that PDT with Pc13 is a promising and non-toxic antitumor modality for melanoma treatment that efficiently induces cell death and reduces tumor growth *in vivo*.

231. (26) **CDU/5-FC SUICIDE GENE BYSTANDER EFFECT IS MEDIATED BY 5-FU AND ENZYME CODING INFORMATION IN HUMAN MELANOMA CELL LINES**

Bernabei-Cornejo SG[1], Páez AV[1], Glikin GC[1], Finocchiaro LME[1]

1. Unidad de Transferencia Genética, Área Investigación, Instituto de Oncología "Ángel H. Roffo", Universidad de Buenos Aires.

Background: The yeast cytosine deaminase::uracil phosphoribosyl transferase (CDU) fusion protein [associated to its prodrug 5-fluorocytosine (5-FC)], was proposed as a suicide gene (SG) therapy approach for human melanoma. Previously, we reported the cytotoxic effects of this system on 4 melanoma cell lines (A375, hM1, hM4, hM9). Here, we extended the study to 4 additional cell lines (hM2, hM10, SB2 and M8).
Objective: To explore the mechanisms enhancing the cytotoxic effects of CDU/5-FC SG system on human melanoma cells.
Methods: Dose-response experiments for 5-FC and 5-fluorouracyl (5-FU) were performed on CDU lipofected and unlipofected cells, respectively. Conditioned medium (CM) from CDU lipofected cells was obtained after 48 h incubation with or without 5-FC. Cell survival was determined by the acid phosphatase assay and proliferative capacity of SG surviving cells by colony formation assay.
Results: A 5-FC concentration dependent decrease in CDU-lipofected cells viability was observed ($p<0.05$). The SG system mimicked 5-FU effects on cell viability. The two SG-resistant cell lines (hM2 and hM10) were also less susceptible to 5-FU. The clonogenic capacity of CDU-lipofected surviving cells was strongly diminished when they were pretreated with 10 μM 5-FC ($p<0.05$) and completely abolished with 100 μM 5-FC or 5-FU ($p<0.01$). Analyzing the contribution of the CDU/5FC-treated cells released factors to the bystander effect, we found that 5-FU accounted for most of the CM citotoxicity. Interestingly, the CDU and/or CDU-coding information was also delivered to the CM. Thus, when CM recipient cells were exposed to 5-FC, it was activated by CDU, resulting in cell death ($p<0.05$).
Conclusion: The CDU/5-FC SG therapy system appears as a promising adjuvant treatment for advanced melanoma. Its bystander effect is attributable to 5-FU and enzyme-coding information released by lipofected cells. Further studies are needed to assess the nature of all the molecule(s) involved.

232. (32) ***IN VITRO* COMPARATIVE ANALYSIS OF HSVTK/GCV AND CD::UPRT/5FC SUICIDE GENE SYSTEMS IN CELL DYNAMICS OF CANINE MELANOMA CELL LINES.**

Paez AV[1], Agnetti L[1], Allende JB[1], Bernabei-Cornejo SG[1], Glikin GC[1], Finocchiaro LME[1].

[1]Unidad de Transferencia Genética, Área Investigación, Instituto de Oncología "Ángel H. Roffo", Universidad de Buenos Aires.

Our group demonstrated the efficacy of herpes simplex virus thymidine kinase/ ganciclovir (HSVtk/GCV) suicide gene (SG) for local control of canine melanoma in a clinical setting.
Aim: As a preclinical study to improve the antitumor efficacy of gene therapy, we explore the *in vitro* effectiveness of the SG system cytosine deaminase/uracil phosphoribosyl transferase fusion enzyme (CDU::UPRT, CDU) and its prodrug 5-FC (5-fluorocytosine; CDU/5FC).
Methods: Prodrug concentration-response experiments were performed on lipofected cells (monolayers and spheroids) in 3 canine melanoma cell lines derived from veterinary patient tumors. Cell viability was measured 5 days post-lipofection by the acid phosphate assay (APH) and clonogenic survival 7 days after reseeding cells by Chrystal Violet staining. Cell death mechanism was determined with acridine orange/ ethidium bromide (AO/EB) and extracellular vesicles (EVs) in the conditioned media (CM) were isolated by 500 xg; 2k xg and 12k xg centrifugation.
Results: The canine melanoma cells were very sensitive to both SG systems in both spatial configurations ($p<0.05$) and surviving cells to both SG treatments showed a significant decrease in their clonogenic capacity ($p<0.001$). AO/EB cell staining showed that during the process of apoptosis, there was an accumulation of vesicles in the perinuclear region and cell periphery. This happened at 24 h in cells exposed to CDU/5-FC, and at 48 h in *HSV*tk/GCV exposed cells. When isolated by differential centrifugation of their respective CM, the 500-2200 nm vesicles were the most toxic fractions from *HSV*tk/GCV cells ($p<0.0001$), while the highest cytotoxicity from CDU/5-FC lipofected cells´ CM was in the fraction containing 30-150 nm vesicles ($p<0.001$).
Conclusions: The present work shows that CD/5FC has a differential effect on cell dynamics and its bystander cell death is mediated by the release of small vesicles and 5-FU, supporting CDU/5-FC as a candidate for a novel *in vivo* protocol.

233. (33) **BRAF INHIBITION DIMINISHES CELL VIABILITY VIA PKC ALPHA (PKCA) IN THYROID CANCER CELLS**

Campos Haedo MN[1], Diaz Flaqué MC[1], Díaz Albuja JA[1], MN, Perona M[2;3], Debernardi MM[1], Cayrol MF[1], Barreiro Arcos ML[1], Sterle HA[1], Juvenal GJ[2;3], Cremaschi GA[1], Rosemblit C[1].

1 BIOMED UCA-CONICET, Buenos Aires, Argentina.
2 Departamento de Radiobiología (CAC), Comisión Nacional de Energía Atómica (CNEA), Buenos Aires, Argentina.
3 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

Thyroid carcinoma (TC) is the most common endocrine neoplasia. Its incidence has increased in the last 40 years worldwide. It comprises a group of tumors of different lineage and biological behavior. About half of TC are driven by an acquired activating mutation in the BRAF oncogene. While targeted therapies have improved outcomes in melanoma patients, most TC patients become resistant or recur suggesting that new or additive non-cross-reactive therapies are needed. We have previously shown that PKCa mediates TSH and thyroid hormones proliferative effects in TC. Recent evidence indicates that together PKCa overexpression and BRAF mutation should contribute to tumorigenesis and resistance to anticancer therapies. We found that by inhibiting BRAF expression with RNAi in anaplastic TC cells with BRAF mutation, PKCa expression decreases as well, suggesting that the latter is found downstream of BRAF. Furthermore, a decrease in the expression of the cell proliferation marker PCNA was observed in BRAF-depleted cells by western blot analysis. Also, TC cells were sensitive to increasing doses of the BRAF inhibitor widely used in the clinic vemurafenib/PLX4032 in a dose-dependent manner ($p<0.0001$) by Cell Titer Blue (CTB) assay. To begin to study the combined inhibition of PKC and BRAF, CTB assays were performed with increasing doses of vemurafenib in presence or absence of the PKC inhibitor GF109203X at selective concentrations in follicular TC cells carrying BRAF mutation. We confirmed the dose-dependency of vemurafenib and found that the combination leads to a significant decrease in cell viability ($p<0.5$). Our results establish that the effective dual PKCa and BRAF blockade can significantly drive tumor proliferation inhibition. The results

obtained could provide new therapeutic targets and alternatives to the treatments currently used for this disease. Despite its increasing incidence and mortality in many cases, TC constitutes a very poorly studied area in our country.

234. (37) **GLYPICAN-3 (GPC3) MODULATES THE ADHESION PROPERTIES OF BREAST CANCER CELLS**

Lugones AC[1], Delgado Pastore M[1], Peters MG[1]
1. Instituto de Oncología Ángel H. Roffo, Área Investigación, Universidad de Buenos Aires.

Glypican-3 (GPC3) is a proteoglycan downregulated in breast tumors. Previously, we showed that GPC3 prevents metastatic spread and regulates the epithelial-to-mesenchymal transition (EMT), suggesting its role as metastasis suppressor. However, events underlying this modulation have not completely described yet.
The aim of this study was to examine the effects of GPC3 on cell morphology and adhesion patterns, as well as on the expression of molecules associated with these properties. We employed human cell lines genetically modified. We silenced GPC3 expression in MCF-7 cells, while it was over expressed in MDA-MB231.
Our results showed that GPC3 expressing cells exhibit an epithelial phenotype and reorganize their actin cytoskeleton. By phalloidin-FITC staining, we observed that GPC3 expressing cells lose their stress fibers and place the actin in a cortical ring. We also checked the expression of linage markers by WB. We found higher levels of the epithelial marker E-cadherin in GPC3 expressing cells, while the expression of the mesenchymal marker vimentin was reduced.
We evaluated whether GPC3 modulates the cell adhesion to extracellular matrix components, showing that it impairs the ability of MDA-MB231 cells to adhere to FN ($p<0.001$, ANOVA Bonferroni´s tests) and LN ($p<0.0001$), as well as to plastic ($p<0.0001$). On the other hand, the GPC3 silencing did not change the adhesion of MCF-7 cells either to FN or plastic, but reduced their adherence to LN ($p<0.001$). We also analyzed the expression of adhesion proteins by WB. Supporting our results, we found that MDA-MB231-GPC3 cells have lower β1 and β4-integrin levels, while no significant changes were found in MCF-7 sublines.
In sum, here we demonstrated that GPC3 modifies several tumor cell properties, like morphology, cytoskeleton organization and adhesion, and modulates proteins related to these processes. Altogether, our results support the key role of GPC3 in the EMT regulation, and then breast tumor progression.

235. (39) **A NOVEL SOLUBLE ISOFORM OF THE HUMAN TGF-β TYPE 2 RECEPTOR EXERTS STRONG ANTITUMOR ACTIVITY IN COLORRECTAL CANCER-DERIVED CELL LINES**

Romo A[1], Guo Y[2], Rodriguez TM[1], Dewey RA[1,3]
[1] Laboratorio de Terapia Génica y Células Madre, Instituto Tecnológico de Chascomús (INTECH), CONICET/UNSAM.
[2] Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong University.
[3] Co-fundador y Asesor Científico de RAD BIO S.A.S.

TGF-β signaling pathway is a key regulator of cancer progression, particularly in colorectal cancer, where 90% of microsatellite instable (MSI) tumors exhibit mutations in the TGF-β receptor type 2 (TGFBR2) gene. Here, we show that lentiviral-mediated overexpression of TGFBR2-SE, a recently discovered soluble isoform of the human TGF-β type 2 receptor, fused to the human IgG1 Fc fragment (TGFBR2-SE/Fc) reduces *in vitro* cell proliferation and migration while induces cell cycle arrest and apoptosis in the primary human colorectal cancer-derived cell line HCT116. Moreover, TGFBR2-SE/Fc impairs tumorigenicity of BALB/c nude athymic mice xenografts, increasing the survival rate of the animals. Tumors overexpressing TGFBR2-SE/Fc were considerable smaller or even unable to be established as only 3 out of 6 mice developed tumors in the TGFBR2-SE/Fc group. Mechanistically, TGFBR2-SE/Fc downregulates TGF-β canonical pathway and leads to the activation of tumor suppressor genes such as p21, p57 and p53, as well as to the inactivation of cell cycle progression elements such as cyclin B1 and Id1. These findings suggest a strong antitumor activity of TGFBR2-SE/Fc based on blocking TGF-β signaling pathway and Smad2/3- independent changes in gene expression supporting the further exploration and development of TGFBR2-SE/Fc as a new biopharmaceutical for the treatment of solid tumors.

236. (42) **VASCULAR NORMALIZATION OF TRIPLE NEGATIVE MAMMARY ADENOCARCINOMAS TREATED METRONOMICALLY WITH CYCLOPHOSPHAMIDE (CY) AND LOSARTAN (LOS)**

Fusini ME[1], Mainetti LE[1,2], Rico MJ[1,2], Kaufman CD[1,2], Grillo MC[1], Guercetti J[1]; Baglioni MV[1,2], Del Giúdice A[1,2], Capitani MC[1], Rozados VR[1,2], Scharovsky G[1,2,3].
[1]Instituto de Genética Experimental-IGE, Facultad de Ciencias Médicas, UNR. [2]CONICET. [3]CIC-UNR, Rosario. Argentina.

CY is an alkylating drug with toxic action on proliferating cells. LOS is an antagonist of angiotensin II receptor, used to treat hypertension. It was postulated that the antiangiogenic effect of metronomic chemotherapy (MCT) could be obtained through a normalization of the abnormal tumor vasculature. Previously, we demonstrated that MCT with CY+LOS, in M-234p and M-406 tumor models, caused inhibition of tumor growth, increase of survival rate and was devoid of toxicity. We aimed to analyze the structural and morphologic changes in M-234p and M-406 vasculature after MCT with CY+LOS. Mice were challenged with each tumor (Day 0). On days 31 (M-234p) and 22 (M-406) tumor samples were taken from: 1) CONTROL: with tumor and no treatment, 2) TREATED: with tumor and treated in the drinking water, from days 5 and 8, respectively, with 2a) CY (25mg/kg/day), 2b) LOS (200mg/kg/day) and 2c) CY+LOS as 2a+2b. Samples were fixed, paraffin embedded, cut in 5µm slices and stained with H&E. The capillaries in CONTROL group showed a circumferentially incomplete inner lining layer of small cells, flattened nuclei, marked intercellular gaps and an underlying sheet of very thin and interrupted connective tissue. No pericytes were observed around the capillaries. Samples from CY+LOS group showed intra and peritumoral capillaries with structure and morphology similar to normal patterns in tissues without tumor. Endothelial cells provided a continuous and uninterrupted linning, with a well-defined basal membrane covered by pericytes. Samples from 2a and 2b tumors were similar to CONTROL group. Results were similar for M-234p and M-406 tumors. The CY+LOS treatment produced modifications of tumor vasculature consisting of normalization of tumor vessels that showed a morphology similar to normal mammary tissue. This changes may reduce hypoxia, increase tumor oxygenation, leading to a better delivery of drugs and a better therapeutic outcome for triple negative mammary tumors.

237. (44) **CHEMOTHERAPEUTIC DRUGS INDUCE THE ACTIVATION OF PROTEINS ASSOCIATED WITH TUMORIGENESIS AND DRUG RESISTANCE IN LOWER-GRADE TUMOR CELLS**

Mayra A. Ríos Medrano *(Instituto de Investigaciones Biomédicas (INBIOMED UBA-CONICET))*, Jesica G. Prada *(Instituto de Investigaciones Biomédicas (INBIOMED UBA-CONICET))*, Ana F. Castillo *(Instituto de Investigaciones Biomédicas (INBIOMED UBA-CONICET))*, Paula M. Maloberti *(Instituto de Investigaciones Biomédicas (INBIOMED UBA-CONICET))*, Ernesto J. Podestá *(Instituto de Investigaciones Biomédicas (INBIOMED UBA-CONICET))*, Ulises D. Orlando *(Instituto de Investigaciones Biomédicas (INBIOMED UBA-CONICET))*

Acyl CoA synthetase 4 (ACSL4) is an enzyme participating in the metabolism of arachidonic acid. ATP-binding cassette (ABC) transporters are transmembrane proteins that translocate low molecular weight molecules through ATP hydrolysis.We have previously shown that ACSL4 is involved in resistance to chemotherapeutic agents by regulating the expression of transporters; thus, the objective of this work was to study the effect of chemotherapeutic agents on ACSL4 and resistance mechanisms. The experimental mod-