tive 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**

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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**

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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)**

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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**

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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 molecu-

lar 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 model consisted in the chemotherapeutic challenge of adrenal cancer NCI-H295R and breast cancer MCF-7 cells, two lines characterized by low aggressive phenotypes and low expression of the ACSL4, ABCG2 and ABCC4 proteins. We evaluated cell functionality using proliferation (BrdU) and viability (MTT) assays, and compound exclusion (efflux) using fluorescent Hoechst 33342. ACSL4 and ABC transporters were evaluated by western blot (WB). NCI-H295R cell treatment with doxorubicin (20 nM) and cisplatin (200 nM) increased the expression of ACSL4 (WB-p <0.001), ABCG2 (WB-p <0.001) and ABCC4 (WB-p <0.05). The treatments also improved fluorescent compound exclusion (efflux-p <0.01), an effect reversed by the action of ABGC2 transporter inhibitor KO143. Combined treatments (chemotherapeutic agents and ACSL4 inhibitor) reduced the proliferation of NCI-H295R cells (BrdU-p <0.05). MCF-7 cell treatment with doxorubicin and cisplatin increased the expression of ACSL4 (WB-p <0.001) and ABCG2 (WB-p <0.05) and the phosphorylation of pAKT (WB-p <0.05) and pS6 (WB-p <0.01), components of the AKT/mTOR pathway. These results are in line with our previous observation that ACSL4 regulates ABGC2 expression through the regulation of the AKT/mTOR pathway. Therefore, ACSL4 may constitute a therapeutic target at the initial stages of chemotherapeutic treatment to prevent the activation of pathways associated with increased tumor aggressiveness.

238. (46) **HEMEOXYGENASE-1 IN THYROID CANCER PROGRESSION**

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Previous work from our group shows that Hemeoxygenase-1 (HO-1) is overexpressed in several types of tumor and the enzyme can be located in cell cytoplasm and/or nucleus. This subcellular distribution is caused by the cleavage of the C-terminus of HO-1 by calpain 1 (CAPN1), calpain 2 (CAPN2), cathepsin B (CTSB) and signal peptide peptidase (SPP). In thyroid cancer (TC), HO-1 potential utility as biomarker remains underexplored. The aim of this work was to study HO-1 expression in TC and its correlation with clinical-pathological data. Tumor biopsies (N=64) and fine needle aspiration biopsies (FNAB) (N=22) were used to asses HO-1 expression by inmunohistochemistry (IHC) and inmunocytochemistry (ICC), respectively. In addition, mRNA expression of HO-1, CAPN1, CAPN2, CTSB and SPP were analyzed by using GEPIA2 and Kaplan-Meier Plotter databases in *in silico* assays. In TC biopsies, overexpression (OE) of HO-1 by IHC was found in the tumor (T) respect to non-malignant areas to the tumor (NMT) (Mann Whitney test, p<0.0001). In T, HO-1 was expressed in the cytoplasm while in NMT, nuclear expression was found. HO-1 expression correlated with histological subtype by IHC (Chi^2, p=0.0006) and Bethesda classification by ICC (Chi^2, p=0.0470). *In silico* studies (ISS) corroborated IHC results in papillary TC (ANOVA, p<0.001). Stage IV female patients with HO-1 OE were associated with lower overall survival (Log rank, p=0.032). ISS showed that stage III male patients with OE of CTSB and female patients with OE of CAPN1 correlated with greater survival (Log rank, p=0.017; Log rank, p=0.027 respectively). However, in female and male stage IV patients, OE of CAPN2 was associated with lower survival (Log rank, p=0.0015; Log rank, p=0.039 respectively). Furthermore, SPP OE correlated with lower survival in female patients (Log rank, p=0.041). So far our results show that HO-1, CAPN2 and SPP overexpression together could be used as unfavorable biomarkers in TC.

239. (64) δ-**TOCOTRIENOL POTENTIATES THE INHIBITORY EFFECTS OF INTERFERON ALFA 2-B (IFN A) ON PROLIFERATION, MIGRATION, INVASION AND INCREASES APOPTOSIS IN HUMAN HUH7 HEPATOCARCINOMA CELLS.**

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Our group has previously postulated that δ-tocotrienol supplementation to interferon alfa (IFN α) therapy can be used as a strategy against liver cancer cells because combined treatment produced growth inhibition and induced apoptosis in SK Hep-1 tumor cells. According to our preliminary results in SK-Hep1 cells, we decided to check if they were repeated in another liver tumor cell line (HuH7), doing additional migration and invasiveness studies.

Cells were treated with 20000 IU/L IFN α and 25 uM δ-tocotrienol, an isomer of vitamin E (combined IFN-E-group). Also, treatments with each single compound were made (IFN-group and E-group). MTT assay was performed to determine cell viability at 72 h of treatment; wound healing assay was done at 24 h to determine cell migration. Invasion studies at 24 h were made in transwell chambers, and annexin v/propidium iodide assay was performed to determine apoptosis at 72 h. As expected, IFN-E-group showed a higher decrease in cell viability (-70%$^{*\#}$) compared with monodrug therapy: IFN-group (-10%*), E-group (-15%*). IFN-E-group displayed a significant decrease (-44%$^{*\#}$) in migratory activity compared with each individual treatment: IFN-group (-21%*) and E-group (-22%*). Also, IFN-E-group showed a significant diminution (-75%$^{*\#}$) in cell invasiveness compared with monodrug therapy: IFN-group (-25%*) and E-group (-55%*). Finally, IFN-E-group showed a higher increase in total apoptosis (+160%$^{*\#}$) compared with individual therapy: IFN-group (-40%*) and E-group (-43%*),(*p≤0.05 vs. control untreated cells; $^{\#}$p≤0.05 vs IFN-group and E-group). In summary, we demonstrate that the addition of δ-tocotrienol to IFN α therapy enhances the reduction of cell proliferation and migration/invasiveness capacities of Huh7 cells, as well as potentiates the increase in apoptotic cell death. In this regard, combined treatment of immunochemicals together with natural products, might open a potential clinical aproach for HCC treatment in the future.

240. (81) **miR-34a AND miR-137 AND THEIR TARGET PROTEINS WERE FOUND TO BE DOWNREGULATED IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS**.

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Acute Lymphoblastic Leukemia (ALL) is the most frequent cancer in children, characterized by clonal proliferation of early B- and T-lymphocyte progenitors. Up to 25% of children and more than 50% of adults suffer a relapse of the disease which significantly reduces patient's survival. Therefore, it is important to identify new biomarkers, which can be used to improve the disease prognosis and/or to predict treatment efficacy. Non-coding RNAs have been shown to play a key role in the development and progression of tumors.