411. (269) **SIGNALING NETWORK INVOLVED IN THE GPC3-INDUCED INHIBITION OF BREAST CANCER PROGRESSION**

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We showed that GPC3 overexpression in breast cancer cells prevents metastatic dissemination, as well as it inhibits canonical Wnt and Akt pathways, while non-canonical Wnt and p38MAPK cascades are activated. However, the hierarchical sequence through which GPC3 modulates these pathways has not been determined.
In this study, we aimed to investigate the mechanism involved in the GPC3 effect on breast tumor progression, focusing on Wnt pathway. We employed the murine mammary LM3 cancer cell line (ER -, PR -, GPC3 -), overexpressing GPC3.
We confirmed by cytoplasmic β-Catenin levels and its transcriptional activity, that GPC3 inhibits autocrine and paracrine canonical Wnt signaling. We demonstrated by qPCR microarrays that GPC3 can modulate Wnt pathway in a genomic way. Out of the 84 evaluated genes, only Wnt 5b (non-canonical) was upregulated, 66 genes were downregulated (several Fz), and the expression of 17 genes -including several Wnt factors- was not modified by GPC3 overexpression (3-fold change, $p \leq 0.05$). Our WB from conditioned media indicated that GPC3 is secreted, suggesting that it competes with Wnt factors and thus prevents their binding to Fz.
In the cross-talk studies, we demonstrated that the GPC3-induced inhibition of Akt is necessary for the non-canonical Wnt activation, and for the canonical inhibition, but it has no effect on p38MAPK. The p38MAPK activation was required for the non-canonical Wnt upregulation and for the canonical Wnt and Akt pathways inhibition. The canonical Wnt blocking was crucial for the Akt downregulation as well as for the p38MAPK and non-canonical Wnt pathways activation. Finally, the non-canonical Wnt activity regulated canonical Wnt and p38MAPK pathways, although it had no effect on Akt.
In conclusion, our data indicate that GPC3 is secreted and it operates through an intricate signaling network. From the balance of these interactions, the inhibition of breast metastatic spread induced by GPC3 emerges.

412. (291) **ISOLATION AND CHARACTERIZATION OF NOVEL MURINE MAMMARY CELL LINES WITH DIFFERENTIATED AGGRESSIVE PHENOTYPE**

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Breast cancer is the first cause of death from female cancer. The recurrence of the disease originated at the level of secondary organs, or metastasis, is responsible for 90% of deaths from cancer. The factors that endow these cells with metastatic functions are largely unknown. One of the limitations in the study of tumor cells with metastatic phenotypes is that cell lines maintained in culture lose this ability to invade and colonize tissues. On the other hand, it has been shown that reinjection of cells in animals can lead to their enrichment with aggressive phenotypes. The aim of this work was the isolation and characterization of different cell populations with differentiated metastatic capacities. Following inoculation of the F3II murine mammary carcinoma cell lines, we established cell populations in vitro, one from the primary tumor and another from a metastatic nodule, F3II TP and F3II NM cell lines respectively. To determine their aggressiveness, a series of additional characteristics were compared between these lines and F3II. The three lines showed variations in morphology in culture and a different doubling time, with F3II NM having the highest one. Moreover, F3II NM presented major adhesion capacity and lower clonogenic potential. This could be explained by the differential expression of cell adhesion molecules, such as integrins or cadherins analyzed by flow cytometry. In addition, the migration capacity was analyzed by transwell assay and the results showed differences in this process. Finally, we compared the behavior *in vivo* and we detected variations in tumor progression such as latency, frequency of ulceration, tumor growth and the presence of pulmonary nodules. All things considered, the establishment and characterization of these two new different cell lines with differentiated metastatic capacities will allow us to determine molecular differences involved in the metastatic process.

413. (326) **INHIBITION OF GLUCOSE METABOLISM POTENTIATES METFORMIN CYTOTOXICITY IN CANINE AND FELINE MELANOMA CELLS**

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Cancer cells exacerbate glucose consumption by increasing not only glycolysis but also pentose phosphate pathway (PPP). Feline and canine melanomas are highly aggressive pathologies that have been proposed as an interesting model of human melanoma. The aim of the present work was to investigate the in vitro effects of a combination of metformin (MET, antidiabetic drug, OXPHOS inhibitor) with 2-deoxyglucose (2DG, glycolysis inhibitor) or 6-aminonicotinamide (6AN, PPP inhibitor) on two melanoma cell lines, Sc (canine) and Dc (feline) derived from spontaneous tumors. Sc and Dc were grown as monolayers at subconfluent cell density. After 24 h of culture, Dc and Sc monolayers were treated with 0.5 or 1 mM 2DG, 2.5 or 1 mM MET and 10 or 25 μM 6AN respectively, or a combination of them. Concentration-response curves were also assessed. The antitumor effects of bioenergetic inhibition were evaluated by the acidic phosphatase assay (APH) 5 days after treatments. We found that both cells lines significantly decreased cell viability ($p<0.05$) in a concentration dependent manner after 2DG and MET treatments whereas only Sc was significantly affected by 6AN. In addition, MET cytotoxic effect was significantly potentiated ($p<0.05$) by the combination of both 2DG and 6AN in both cells lines. We further analyzed the mechanism involved in the effectiveness of both combinations by means of specific fluorescent probes (Flow cytometry). We found that MET/2DG and MET/6AN increased intracellular oxidants (DCF, $p<0.05$) and acidic vesicles (NA, $p<0.05$) thus suggesting an autophagic process. The results reported here support further studies to investigate the potential use of this metabolic modulation approach in a clinical setting.

414. (346) **MECHANISM INVOLVED IN THE SYNERGISTIC EFFECT OF METFORMIN AND G6PDH INHIBITION**

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Most cancer cells display a strikingly different metabolism than normal cells. Cancer cells exacerbate not only glycolysis (known as the "Warburg effect") but also TCA cycle and PPP to enhance the malignant phenotype. Previously, we described a synergic cytotoxic effect on eight melanoma cell lines by using metformin (MET, an inhibitor of OXPHOS complex I and an AMPK´s indirect activator) in combination with 6-aminonicotinamide (6AN, an G6PDH inhibitor). The aim of the present work was to determinate the effect of this combination on metabolic parameters and to elucidate the mechanisms involved. We used as study model three melanoma cell lines, hM1 (BRAFV600E) and hM4 (BRAFV600R) established from tumor of IOAHR´s patients and A375 (BRAFV600E) a commercial cell line. As metabolic parameters we evaluate extracellular glucose and lactate. Melanoma cells treated with 6AN and MET/6AN showed a lower glucose consumption and lactate production than cells treated with MET or control cells. Also, we determined the reductive power which decreased in 6AN treated cells. To elucidate the mechanism involved, we measured the intracellular oxidants (DCF-DA probe) and mitochondrial membrane potential (TMRM and Mitotracker CMXRos probe). At short term MET/6AN displayed an increase of mitochondrial depolarization and intracellular oxidants. In contrast, long term survival cells exhibited mitochondrial hyperpolarization. Also, we could determinate a rise on intracellular complexity (SSC) on cells treated with the combination MET/6AN. On the other hand, we evaluated apoptosis and necrosis using two techniques Annexin

V-Propidium Iodide and Orange acridine-Ethidium bromide staining. The combination MET/6AN exhibited a strong rise on late apoptotic or necrotic events. Also, we found an increase on Sub G0 population although we did not detect a change on the cell cycle. Our results reported here support further studies to investigate the potential use of this metabolic modulation approach in a clinical setting.

415. (389) **EFFECT OF THYROID HORMONES ON THE ACTIONS OF ONCOLOGY DRUGS IN BREAST CANCER CELLS.**

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Chemoresistance is a major cause of cancer treatment failure. Many breast cancer cells acquire multidrug resistance (MDR) by upregulating the level or activity of membrane protein such as Pgp, which enable the exclusion of cytotoxic substances from the intracellular environment. Previously we demonstrated that Thyroid Hormones (THs) modulate CYP3A4 expression and Doxorubicin chemosensitivity in T lymphoma cells. However, in breast cancer cells, little is known about these mechanisms that lead to tumor chemotherapy resistance and are crucial to assure the success of treatment. Bexarotene and Lapatinib are recommended for breast cancer treatment but thyroid disfunction, is recognized as an important side effect of such therapies, potentially manageable by TH administration. Being MDR1 the major protein involved in the efflux of cytotoxic agents, we reasoned that Bexarotene- and Lapatinib-induced MDR1 activity may act as an important regulator in breast cancer MDR and that THs could modulate these effects. To this end, we first demonstrated that triple negative breast cancer MDA-MB-231 cells display both TRβ and integrin avb3 dimer (TH membrane receptor). Also, we demonstrate that Bexarotene and Lapatinib inhibits MDA-MB-231 cells proliferation ($p<0,05$) and THs increased cell viability ($p<0.01$) and impaired the action of both drugs. On the other hand, Bexarotene and Lapatinib modulate MDR1 mRNA expression and protein levels. We also evaluate MDR1 activity and found that both bexarotene and lapatinib induce Rho123 exclusion, but TH treatment did not revert this effect. In conclusion, THs affect the action of oncology drugs by mechanisms to be studied, but that would not include the participation of MDRs.

416. (416) **ROLE OF THE PRO-APOPTOTIC FACTOR NOXA IN THE SENSITIVITY OF PATIENT-DERIVED GLIOMA STEM CELLS AGAINST CHEMOTHERAEPUTIC AGENTS**

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Glioblastoma multiforme is one of the most malignant types of central nervous system tumors. Despite advances in treatments it remains largely incurable. This recurrence is attributed to the presence of a highly resistant subpopulation of tumor cells named glioma stem cells. Defective or inefficient apoptosis is an acquired hallmark of cancer cells. Thus, a thorough understanding of apoptosis resistance mechanisms is imperative to unravel novel drug targets for the design of more effective therapies. The BH3-only proteins of the Bcl-2 family can trigger apoptosis by binding to the pro-survival members of this family and neutralizing their activity. This concept has prompted the development of small molecules capable of mimicking BH3-only proteins leading to apoptosis, and thus, sensitizing cancer cells to treatments. Herein, we exposed five patient-derived glioma stem cell lines to routinely used chemotherapeutic drugs (temozolomide, lomustine and vincristine) and BH-3 mimetics (ABT-263 and WEHI-539). Viability assays revealed that the combination of BH3 mimetics that target Bcl-xL with chemotherapeutic drugs led to a marked increase in cell death compared to that triggered by each drug alone. Notably, one cell line resulted particularly sensitive to these combination therapies and this sensitivity correlated with the expression of the BH3-only protein NOXA. ABT-263 not only increased the degree of cell death but also induced NOXA mRNA levels as judged by RT-qPCR analysis. Moreover, we observed that siRNA-mediated downregulation of NOXA protected glioma stem cells from BH3-mimetic-induced cell death. These results indicate that NOXA contributes to glioma stem cell apoptosis and that its expression could represent a predictive biomarker of sensitivity to Bcl-xL inhibitors. Therefore, a proposed strategy to combat brain tumors that express NOXA would consist of combining Bcl-xL inhibitors with agents that potentiate NOXA activity.

417. (544) **HUMAN CLEAR CELL RENAL CELL CARCINOMA: HLA-G EXPRESSION AND MICROVESSEL DENSITY ANALYSE.**

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Renal cell carcinomas (RCCs) are the third genitourinary malignancie, behind prostate and bladder carcinoma. The most aggressive and deadly subtype is the clear cell RCC (ccRCC). The habitual oncological treatments are radio and chemotherapy, but there is a high percentage of failure in this treatment. New strategies are being tested, like immune and angiogenesis therapy, but results are controversial. In this context, the HLA-G molecule appears as a new perspective of anti-tumor therapeutic strategy, since generates suppression and tolerance of the immune system and is expressed abnormally in some types of cancer as a mechanism of immune evasion. On the other hand, microvessel density (MVD), determined by immunohistochemical staining with CD34, is used as indicator of neoformation of sanguineous vessels. Our aim was to create a primary cell culture, using human samples from partial or radical nephrectomies to evaluate MVD and HLA-G expression. Methods: MVD was determined by CD34 staining in the tumor samples and HLA-G expression was determined by real time-PCR from cells grown from primary cell culture. Preliminary results showed that all tumor samples are HLA-G positive, but this expression is not homogeneous. Some samples expressed HLA-G only in the peripheral tumor area, others only in the central area, and others in both. No patient expressed HLA-G in the normal renal parenchyma surrounding the tumor. MVD index was observed to be higher in the peripheral tumoral area than in the central one. We can conclude that the intratumoral heterogeneity observed in the HLA-G expression as much as CD34 expression could be the reason for therapeutic failure. These knowledge, about ccRCC, is important not only for new target therapy but also to improve the diagnosis and prognosis of this important neoplasia.

418. (554) **FUNCTIONAL DIFFERENCES BETWEEN METASTASIC AND NON-METASTASIC OSTEOSARCOMA CELLS AND DIFFERENT POTENCIAL IN THEIR CAPACITY TO INDUCE DIFFERENTIATION**

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Osteosarcoma (OS) is the most common bone malignant tumor, affecting mainly children and young adults. Lung metastasis is a therapeutic challenge during osteosarcoma progression (15–30% survival rate with pulmonary metastasis at diagnosis). Niche establishment is critical for metastasis. Through proteomic analysis we demonstrated differential gene expression related to molecular function between metastasic OS (LM7) and non-metastasic (SAOS2) OS cell lines. Molecular differences were reflected in variations in the differentiation capacity in the two OS cell lines that differ in their