were challenged orthotopically with M-406 tumor (day 0); when the tumor was palpable, mice of SD and HFD diets were distributed into 2 groups, GI: Control, with no treatment; GII: Cy 25mg/kg/day+Los 150mg/kg/day in the drinking water. Mice were weighted and tumor volume measured 3 times/week. When tumors were exponentially growing, mice were euthanized, tumors excised, fixed and paraffin included. *Peritumoral* and *intratumoral* macrophages [Total-Mt-(F4/80$^+$), M1 (iNOS$^+$, antitumoral) and M2 (MRC1$^+$, protumoral)] were analyzed by immunofluorescence. GI-HFD mice showed a significantly higher number of Mt and M2 *peritumoral* macrophages compared to GI-SD mice ($P<0.05$). MCT with CY+LOS produced a significant increase in Mt and M1 peritumoral macrophages in both dietary groups when compared to the respective controls ($P<0.05$). The *intratumoral* macrophages of HFD mice showed a significantly higher number of Mt and M1 than that observed in GI-SD mice ($P<0.05$). Interestingly, MCT with CY+LOS produced an increase of Mt and M1 macrophages, only in the SD group. Conclusion: HFD increase protumoral macrophages in the invasive front of the tumor and MCT with CY+LOS reverts such an effect, increasing M1 macrophages. HFD has a deleterious effect on the M1 polarization of the MCT with CY+LOS, which would explain its lower antitumor effectiveness, as shown previously. Hence, the comorbidity impairs the treatment effect.

559. (575) **TARGETING OF MITOCHONDRIAL PEPTIDE HUMANIN TO IMPROVE CHEMOSENSITIVITY IN GLIOBLASTOMACELLS**

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Humanin (HN) is a mitochondrial peptide with a robust cytoprotective many cell types. HN can interact with proteins of the bcl-2 family or be released and bind with two membrane receptors: a trimetric receptor, and the FPR-2 receptor. HN protects normal tissues from chemotherapy, and the administration of HN analogs has been proposed as a therapeutic approach for degenerative diseases. However, its role on the pathogenesis of cancer is poorly understood. Here we aimed to evaluate whether HN affects chemo-resistance of glioblastoma (GBM) cells. We first assessed the effect of chemotherapy on HN expression in murine (GL26) and human (U251) GBM cell lines, as well as in primary cultures from GBM biopsies. By immunofluorescence we observed that cisplatin upregulates HN in all the cells evaluated. To analyze the effect of HN on chemotherapeutic cytotoxicity, we used a HN analog peptide (HNG). In human GBM cells we observed that HNG abolished the cytotoxic and antiproliferative effect of cisplatin, restoring viability and clonogenic capacity (Two-way ANOVA $p<0.05$). Blockade HN interaction with the FPR-2 receptor, using a specific antagonist (WRW4), limited the cytoprotective function of both endogenous and exogenous HN in human GBM cells exposed to cisplatin, as assessed by MTT assay and BrdU incorporation (Two-way ANOVA $p<0.05$). To explore the effect of endogenous HN on GBM cell chemosensitivity, we developed a baculoviral vector encoding a HN-specific shRNA for the transcriptional silencing of its expression. These vectors showed excellent transduction efficiency in these cells. We observed that the inhibition of endogenous HN exerts an inhibitory effect on the viability of GBM cells and increases their sensitivity to cisplatin. Our study suggests that HN favors chemoresistance in GBM cells and that it could hold value as a therapeutic target to improve their response to conventional treatment.

560. (579) **METRONOMIC PHOTODYNAMIC THERAPY *IN VITRO* EVALUATION FOR MALIGNANT GLIOMAS**

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Glioblastoma (GBM) is the most aggressive brain tumor. New therapies are proposed such as Photodynamic Therapy (PDT) that combines light, oxygen and photosensitizers (PTs) to overcome conventional treatment issues. An important disadvantage of PDT using high light flux rates (PDTc) is the abrupt oxygen consumption, which leads to resistance to the treatment. PDT metronomic regimens (PDTm) administering light at low irradiation intensity could be an alternative to validate PDT for GBM. The main objective of the present work was to compare PDT effectiveness with conjugated polymer nanoparticles (CPN) in two modalities: PDTc or PDTm based on cell viability, impact in tumor microenvironment modulation and HIF-1α activation as indicator of oxygen consumption. To accomplish this, GBM cell lines U87MG y T98G and THP-1 macrophages were used. PDT efficacy was assayed in GBM mono-culture and co-culture with macrophages using 10 and 40 J/cm^2 with 84 mW/cm^2 and 17 mW/cm^2 for PDTc and PDTm respectively. In order to determine the activation of HIF-1α, GBM MO59K cells genetically-modified to overexpress a GFP-associated with the hypoxia response element, where HIF binds, was used. GBM were incubated with CPN (3 and 6 ug/mL), then irradiated until reaching 10 J/cm^2 in both modalities, and GFP expression was measured by flow cytometry. At 6 and 24 h after PDT, PDTc increased HIF-1α activation regarding PDTm. Cell viability was also dissimilar between PDTc and PDTm at the same CPN concentration and light doses (10 and 40 J/cm^2). T98G cells were more resistant in both modalities and cell viability decreased significant using PDTm (16,1;13,8;15,6; and 31,40 % with 47,5, 23,75, 11,875 y 5,93 ug/mL CPN) compared to PDTc (19,4, 35,8, 36,9% y 72,3% corresponding to CPN concentrations of 47,5, 23,75, 11,875 y 5,93 ug/mL) in U87MG. PDTm resulted in a more pronounce cell death with less HIF-1 activation as a main resistant molecular mechanism triggered proposing this modality as most suitable for GBM treatment.

561. (580) **ANALYSIS OF PLAGL1 EXPRESSION IN THYROID CARCINOMAS**

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The PLAGL1 gene encodes for a zinc finger transcription factor proposed to act as a tumor suppressor involved in cell cycle arrest and apoptosis. However, PLAGL1 is overexpressed in some neoplasms suggesting an oncogenic function. Our aim was to study PLAGL1 expression in Thyroid Carcinomas (TC) and the association between high PLAGL1 levels and poor prognosis. PLAGL1 expression was evaluated by IHC in human thyroid tissue samples and by RT-PCR and Western blot on a large panel of human thyroid cell lines. For transcriptomic analysis we downloaded datasets from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) data from papillary thyroid carcinomas (PTC). DNA methylation analysis and clinical characteristics were obtained from the

PTC-TCGA dataset. We detected the expression of the large and short isoforms of PLAGL1 in different TC cell lines. Furthermore, in TC samples, we identified cytoplasmic and nuclear expression of PLAGL1. Transcriptomic analysis indicated a significant (P<0.0001) downregulation of PLAGL1 in differentiated and poorly differentiated carcinomas compared with nonneoplastic thyroid tissue, while PLAGL1 overexpression (P=0.003) was observed in anaplastic carcinomas. We have found no association between PLAGL1 expression levels and the methylation status on their promoter (P=0.09). In PTC-TCGA database, we found two subtypes of tumors that displayed distinct PLAGL1 levels (P<0.0001). PTC with high PLAGL1 expression tend to have a higher degree of malignancy regarding shorter disease-free survival (P=0.016), association with BRAF mutations (P<0.0001), increased dedifferentiation (P=0.046), advanced T stage (P=0.03), a greater number of lymph node metastases (P=0.03), and a strong correlation with the number of M2 macrophages (P<0.0001).Here we describe an association between high PLAGL1 expression and an aggressive phenotype of thyroid tumors suggesting that this transcription factor might serve as a cancer risk predictor.

562. (590) **GENETIC TESTING OF FINE-NEEDLE ASPIRATIONS FOR DIAGNOSIS OF THYROID CANCER**

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Introduction

The frequency of palpable thyroid nodules is ~5% in the population, but only a small fraction of them are malignant (thyroid cancer). Fine-needle aspiration (FNA) with cytological evaluation is the most reliable tool for cancer diagnosis in thyroid nodules. However, ~20% of nodules are diagnosed as indeterminate by cytology, making it difficult to optimally manage these patients. The introduction of multigene molecular panels has improved the diagnostic accuracy among indeterminate thyroid nodules. Objectives: We sought to develop a multigene molecular panel to conduct genetic testing to improve the FNA diagnosis of thyroid nodules. Methods: We developed a PCR-based multigene molecular panel consisted of BRAF codons 600 and 601, H/N/KRAS codon 12, 13 and 61 point mutations (Sanger sequencing), and several RET, NTRK, ALK, BRAF and PPARγ rearrangements (multiplex PCR). Results: Genetic testing was conducted in a pilot retrospective study including 10 FNA samples with cytology suggestive of malignancy (Bethesda V). We purify genomic DNA and total RNA from residual material from FNA samples. The point mutations p.V600E BRAF (n=6) and p.G12V HRAS (n=1), and the RET/PTC1 (n=2) and ETV6-NTRK3 (n=1) rearrangements were detected. The presence of mutation was a strong indicator of cancer because anatomical-pathological analysis after surgery indicated thyroid cancer in all mutation-positive nodules. Conclusion: A combination of cytology and clinically applicable genetic testing showed significant advance in the diagnostic accuracy of malignancy in the nodules, improving presurgical malignancy risk assessment in FNA in order to avoid unnecessary diagnostic surgeries. Moreover, genetic testing allow to design personalized therapy specific to the needs of individual patients with thyroid cancer.

563. (591) **ROLE OF ASCL1 IN NEUROBLASTOMA TUMORIGENICITY**

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Neuroblastoma (NB) is the most common solid extracranial pediatric cancer derived from the sympathoadrenal lineage. Ascl1 is a proneural transcription factor whose transient expression is essential for the development of this lineage. Ascl1 remains overexpressed in NB cells and its high expression is associated with poor clinical prognosis. The aim of this work was to evaluate the role of Ascl1 in the tumorigenicity of NB cells. We evaluated the phenotype of Ascl1 knockdown (KD) SK-N-SH NB cells relative to control and analyzed scRNA-seq data from patients' tumors to understand NB tumor cell heterogeneity. By performing a Trypan Blue assay, we found that Ascl1 KD does not affect cell viability. Analysis of proliferation by clonogenic assay and immunohistochemistry against Ki67 showed that the KD of Ascl1 significantly reduces the proliferating capacity of NB cells. Moreover, Ascl1 KD cells exhibited changes in their morphology relative to control, showing a neuron-like shape. For scRNA-seq analysis we obtained transcriptomic data of human NBs from public repositories. We integrated the data into a single dataset, performed dimensional reduction and clustering, and analyzed the origin of the cells. We characterized the resulting clusters through analysis of functional enrichment and analyzed expression of Ascl1 and co-expressed factors using non-negative matrix factorization. We found that NB cells share a single origin: sympathoblasts of the adrenal medulla, that cellular heterogeneity can be explained by the phase of the cell cycle, and that Ascl1 is expressed across all cell types in association with neurogenesis-related genes. The obtained results show that: a) Ascl1 blocks terminal neuronal differentiation in NBs, supporting tumor progression, b) a therapy against Ascl1 could target several tumoral subpopulations and, together with the observed effects of the Ascl1 KD *in vitro* sets the basis for a therapeutic strategy based on the modulation of Ascl1.

564. (601) **sTn EXPRESSION IN EXTRACELLULAR VESICLES DERIVED FROM BREAST CANCER PATIENTS**

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Breast tumors may secrete extracellular vesicles (EVs) of varying sizes, which may present on their surface O-glycans related to immune evasion or the spread of tumors. The sialyl antigen Tn (sTn) is the result of the early addition of sialic acid to the core of Ser/Thr-GalNAc, which disrupts the elongation of the O-glycan chain and in turn associates it with greater aggressiveness in various adenocarcinomas. To analyze the expression of sTn in breast cancer EVs, 27 patients with stage I and II tumors were analyzed by immunohistochemistry and EVs were isolated from the serum of these patients and four controls. Differential centrifugation followed by size exclusion chromatography (SEC) in CL2B agarose columns was used to isolate EVs. The morphology and size of the EVs were verified by dynamic light diffraction (DLS) and electron microscopy (ME); the protein content was determined by Qubit assay and by calculating the absorbance at 330 and 260/280 nm for each fraction. The presence of the markers CD9 and CD63, as well as sTn in the fractions was analyzed by dot blot; positive fractions were concentrated and analyzed by Western blot (WB). The SEC fractions showed that 11 of 27 patients were positive for CD63 (40.7%) and 13/27 (48.1%) for CD9 and 7/27 (25.9%) for sTn. Only 1 in 4 patients positive for sTn in the primary tumor was also positive in the fractions from serum EVs. All sTn positive fractions were positive for CD9 and CD63 and showed small-sized EVs using ME and DLS (97 nm mean). WB analysis showed low and high molecular weight bands in the range of 60 to 120 kD. No relationship was found between the expression of sTn and the pathological variables of the patients. In conclusion, it was possible to detect the presence of sTn in EVs derived from few patients with breast cancer.

565. (618) **MICROBIOME RELEVANCE IN TUMOR TISSUE OF EARLY BREAST CANCER PATIENTS**

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