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Comparison of Proliferation Marker Expression in Napabucasin and APX2009 Treated Malignant Peripheral Nerve Sheath Tumors

Biology - Time: Mon 3:00pm-4:00pm - Session Number: 2643
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Emily Hulsey

Malignant peripheral nerve sheath tumor (MPNST) is an aggressive soft tissue sarcoma that affects the nerves, starting in the major nerve trunks. MPNST accounts for 5-10% of all soft tissue sarcomas. MPNST usually occurs between 20 and 50 years of age and occurs in 1 in 100,000 people. Around 50% of all MPNSTs occur in people with neurofibromatosis-1. These tumors are highly refractory to treatment

New data suggests STAT3 as a driver in the MPNST phenotype[1]; we tested the efficacy of Napabucasin (Napa) a reported STAT3 / stemness inhibitor[2] in MPNST tumors. Napa is a cancer stem cell inhibitor with potential antineoplastic activity. APX2009 is a second generation inhibitor of the redox activity of APE1 and exhibits potent antitumor activity. The MPNST cell line ST88-14 was implanted into mice and treated with both reagents.

We evaluated the effect of Napa on tumor growth and proliferation. The tumors were stained with cell proliferation markers, Phosphorylated Histone H3 (pH3) and Ki67 using the DAKO Flex system. pH3 stains cells in the late G2 phase and mitosis while Ki67 stains cells in any stage of the cell cycle except for G0.

Slides were imaged with the Aperio Scanscope and analyzed with the Aperio ImageScope system using the positive pixel algorithm to determine the expression of the markers.

Napa and APX2009 both decreased tumor volume with a corresponding decrease in tumor weight at the end of study. In the Napa-treated tumors, Ki67 was significantly decreased and pH3 increased significantly. IHC staining in the APX2009 tumors had expected decreases in proliferation in pH3 and Ki67. Although the tumor growth reduction was significant, we did not observe complete regression indicating combination therapy may be necessary. In the future, we will use orthotopic or PDX models to look at relevant combination therapies to pair with Napa treatment.

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Expression of CD56 in glioblastoma treated with NK cell immunotherapy

Biology - Time: Mon 3:00pm-4:00pm - Session Number: 2644
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Madelynn Gaines

Glioblastoma (GBM) is a rare and aggressive brain tumor with a life expectancy around 14 months. Immunotherapy is a novel approach to fighting cancers that have poor results with chemotherapy, such as GBMs. Natural killer (NK) cells are of great interest for immunotherapy because they are innate cells that do not require prior sensitization in order to induce apoptosis in cancer cells. There are two subtypes of NK cells: $CD56^{bright}$ and $CD56^{dim}$. $CD56^{dim}$ NK cells are the more abundant and cytotoxic subtype. In this study, NK cell therapy was administered intravenously to a GBM mouse model. Radiological analysis included multimodal imaging using MRI T1CE, T2, and 18F-FET PET images to measure tumor volume. All methods showed a lower GBM volume with treatment when compared to control, and T1CE showed statistical significance ($p<0.01$). Histological analysis was done to determine the location of NK cells and to identify tumor infiltration; CD56 immunohistochemistry was used to visualize the subtypes of NK cells. Digital images of the slides were generated using Aperio ScanScope. Image analysis involved the development of a cytonuclear algorithm using Indica Labs HALO software to recognize staining intensity and quantify cells positive for CD56 expression. Our findings included a higher number of weakly stained cells in the NK therapy group than the control group, which may suggest the presence $CD56^{dim}$ NK cells in the GBM. However, we cannot confirm the presence of NK cells due to nonspecific staining throughout the tumor.

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The Role of Carbonic Anhydrase IX in Acute Myeloid Leukemia and Development of a Potential Therapeutic Target

Biochemistry - Time: Mon 1:30pm-2:30pm - Session Number: 2130

Austyn Colter, Callista Maguire, and Dr. George Sandusky, Department of Pathology, Indiana University School of Medicine, 340 W 10th St, Indianapolis, IN 46202 Dr. Heiko Konig and Dr. Mircea Ivan, Department of Medicine, Division of Hematology/Oncology, Indiana University School of Medicine, 340 W 10th St, Indianapolis, IN 46202

Austyn Colter

Acute myeloid leukemia (AML) is a rapidly growing form of blood cancer that affects approx. 20,000 patients in the US per year. Despite achieving a remission in response to intensive chemotherapy, most patients relapse and eventually die from their disease. Evidence suggests that this poor outcome is due to the survival of drug resistant AML cells in O2-deprived niches in the bone marrow (BM). In this study, RNAseq and PCR were employed to identify a potential marker to track hypoxic survival of leukemic cells. Characterization of gene expression was assessed using RNAseq; and Quantitative PCR reactions were performed using PowerUp SYBR GreenPCR Master Mix (Applied Biosystems, USA) on 7900HT Real-Time PCR System. Xenograft models of human AML were treated with varying doses of Cytarabine. In addition, BM from AML patients with drug-resistant disease were assessed for morphologic changes following chemotherapy. Human and mouse tissues underwent staining for Carbonic Anhydrase (CA)-IX and -XII via immunohistochemistry (IHC). Immunostaining was done by the Dako-Flex system platform. The Aperio Whole slide digital imaging system was used to create a digital image, and then computer-assisted morphometric analysis (positive pixel algorithm) was used to analyze the digital images. Our genomic studies demonstrated that CA-IX is the leading CA isoform expressed in AML cells under hypoxic stress conditions. These findings were in line with the results from our xenograft studies, where CA-IX staining was enhanced in leukemic cells remaining after chemotherapy. In patient cases, the blast count directly correlated with CA IX expression. We conclude that (i) due to its membrane-bound location, CA-IX represents a suitable therapeutic target and that (ii) inhibition of CA-IX might represent a useful approach to target drug resistant AML cells that reside in hypoxic environments, such as the BM niche.

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