TAMI-46. FRIEND AND FOE: RADIATION THERAPY INCREASES GLIOBLASTOMA IMMUNE EVASION VIA EVS

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Glioblastoma (GBM) is the most common primary malignant brain tumor and despite optimal treatment, long-term survival is extremely rare. Radiation therapy (RT) leads to successful initial tumor regression but recurrence is inevitable. Previous studies have shown that ionizing radiation leads to a change of immune-related markers on tumor cells. Extracellular vesicles (EVs) are membranous structures secreted by nearly every cell and have been shown drive GBM progression by acting as multifunctional signaling complexes. Here, we show that radiation of GBM cells leads to an altered EV secretion/uptake dynamic, composition, and protein content. Furthermore, we show that EVs secreted by radiated GBM cells modulate the innate (microglia/macrophages) as well as adaptive (T-cells) immune response in the tumor microenvironment. We dissected a novel mechanism by which GBM evades the immune system via EVs following RT, pointing towards novel therapeutic strategies to prevent GBM recurrence.

TAMI-47. MIR-542-3P CONTRIBUTES TO THE HK2-MEDIATED HIGH GLYCOLYTIC PHENOTYPE IN HUMAN GLIOMA CELLS

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BACKGROUND: The elevation of glucose metabolism is linked to high-grade gliomas such as glioblastoma multiforme (GBM). The high glycolytic phenotype is associated with cellular proliferation and resistance to treatment with chemotherapeutic agents in GBM. MicroRNA-542-3p (miR-542-3p) has been implicated in several tumors including gliomas. However, the role of miR-542-3p in glucose metabolism in human gliomas remains unclear. METHODS: We measured the levels of cellular proliferation in human glioma cells. We measured the glycolytic activity in miR-542-3p knockdown and over-expressed human glioma cells. We measured the levels of miR-542-3p and HK2 in glioma tissues from patients with low- and high-grade gliomas using imaging analysis. RESULTS: We show that knockdown of miR-542-3p significantly suppressed cellular proliferation in human glioma cells. Knockdown of miR-542-3p suppressed HK2-induced glycolytic activity in human glioma cells. Consistently, overexpression of miR-542-3p increased HK2-induced glycolytic activity in human glioma cells. The levels of miR-542-3p and HK2 were significantly elevated in glioma tissues of patients with high-grade gliomas relative to that in low-grade gliomas. The elevation of HK2 levels in patients with high-grade gliomas were positively correlated with the high levels of miR-542-3p in GBM and low-grade gliomas (LGG) based on the datasets from the Cancer Genome Atlas (TCGA) database. Moreover, the high levels of miR-542-3p were associated with poor survival rate in the TCGA database. CONCLUSIONS: miR-542-3p contributes to the HK2-mediated high glycolytic phenotype in human glioma cells.

TAMI-48. THE KETOGENIC DIET IS INEFFECTIVE IN PRECLINICAL MODELS OF IDH1 WILD-TYPE AND IDH1 MUTANT GLIOMA

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Despite decades of intensive research, infiltrative gliomas are still usually lethal and challenging to treat. A subset of gliomas contains mutations in *isocitrate dehydrogenase 1* ($IDH1^{mut}$), which disrupts cellular biochemistry; such gliomas are generally less aggressive than their IDH1 wild-type ($IDH1^{wt}$) counterparts. Some preclinical studies have suggested that a ketogenic diet (KD), characterized by low-carbohydrate and high-fat content, may be beneficial against a variety of cancers, including gliomas. However, not all studies have shown promising results, and to date, no study has addressed the sensitivity of glioma cells to KD in the specific context of the endogenous $IDH1^{mut}$ metabolic landscape. The aim of the current study was to compare the effects of KD in preclinical models to $IDH1^{wt}$ versus $IDH1^{mut}$ gliomas. *In vitro* treatment of patient-derived $IDH1^{wt}$ and $IDH1^{mut}$ glioma cells with the ketone body β-hydroxybutyrate showed no significant effect on cell proliferation in a low glucose culture environment. Likewise, the *in vivo* flank growth rates of these patient-derived $IDH1^{wt}$ and $IDH1^{mut}$ glioma xenografts showed no significant difference when mice were fed KD versus regular diet (GBM12 p=0.98, GBM164 p=0.4, GBM196 p=0.11). Finally, KD had no effect on the survival of mice engrafted with isogenic Sleeping-Beauty transposase-engineered $IDH1^{wt}$ (median control survival 22 days versus treatment 23 days, p=0.23) or $IDH1^{mut}$ glioma cells (median control survival 26.5 days versus treatment 26 days, p=0.81). These data suggest that $IDH1^{mut}$ gliomas are not more responsive than $IDH1^{wt}$ gliomas to KD, and that clinical trials further exploring KD in this subset of glioma patients are probably not warranted.

TAMI-49. JAK STAT INHIBITION REVERSES MYELOID CELL INDUCED ANTI TUMOR IMMUNITY IN T CELLS

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BACKGROUND: Many central questions about the immunosuppressive microenvironment in glioblastoma (GBM) remain unanswered, particularly the interaction with lymphoid and myeloid populations. Here, we combined single-cell (scRNA) and spatial transcriptomics (stRNA) to comprehensively characterize the immune interaction with GBM. MATERIAL AND METHODS: We performed scRNA-Seq of 50k CD45+ cells (8 patients) and inferred transcriptional programs and fate decisions in T cells. A novel algorithm (Nearest functionally connected neighbor) was used to predict interacting cells, further validated using spatial transcriptomics and immunofluorescence. Our findings were validated in our human neocortical glioblastoma model with autografted T cells. RESULTS: Integration of st/scRNA-seq revealed a transcriptional shift of T cells towards exhaustion/hypoxia induced dysfunction. Pseudo-time analysis revealed increased Interleukin 10 (IL10) response during the T cell transformation from the effector to the exhausted state. Using NFCN we identified a subset of HMOX1+ myeloid cells (STAT/HMOX axis), responsible for this IL10 release. Computational findings were validated using our human neocortical glioblastoma model with autografted T cells, where IL10R-inhibition/myeloid cell depletion prevented T cell exhaustion/dysfunction ($p < 0.01$) . In order to target the STAT3/HMOX1 axis we used a JAK/STAT inhibitor in our model which showed a drastic reduction of IL10 release ($p< 0.02$) and concordant activation of T cells. Clinically, one patient treated with a JAK/STAT-inhibitor in a neoadjuvant setting, 4 weeks prior to the recurrent GBM surgery, led to a significant increase ($p< 0.001$) in effector T cell population. CONCLUSION: Our findings suggest that targeting the myeloid compartment of GBM provides an opportunity to convert a "cold" into "hot" immune environment which might be helpful to improve all T cell based therapies in the future.

TAMI-50. PATHWAYS ACTIVATION IN GL261 MICE GLIOMA CELLS BY HIV-1 GLYCOPROTEIN GP120

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Patients infected with human immunodeficiency virus type 1 (HIV-1) are more prone to developing cancers, including glioblastomas (GBMs). The median survival for GBM patients with HIV is significantly shorter than for HIV-negative GBM patients, even though they receive the same treatments. This difference indicates that HIV infection is associated with more aggressive tumor behavior and with treatment resistance. Earlier we demonstrated that gp120, a main glycoprotein in the HIV shell, stimulates glycolysis and protein synthesis in glioma cells. The purpose of this study was to evaluate the underlying gp120 dependent signaling mechanism in glioma cell. Mouse glioma cells GL-261 were continuously cultured for 7 days in medium with and without soluble gp120 Bal III (100ng/ml) and collected for Western blot and Cell cycle assays. Western blot analysis presented an increase in phosphorylation of Proline-rich tyrosine kinase (Pyk2(Y402)), p38(YT100/Y182) and p70s6(T421/S424), the key proteins of the Pyk2 pathway, along with the increased levels of Akt(S473) and Glycogen Synthase Kinase 3b (GSK3b (S9)) phosphorylation. Flow cytometry analysis of Cell Cycle revealed an increase of G2/M phase in cells cultured in gp120 Bal III when compared to control cells. Furthermore, GL-261 cells with knockout of Pyk2 via CRISPR Cas 9 gene editing showed no significant change in cell cycle regulation when cultured with gp120 Bal III.Overall, these results demonstrate that gp120 triggers activation of Pyk2/MAPK and Akt/GSK3b pathways and alter cell cycle regulation in GBM. This research was made possible by NIH grant number 1SC1GM122691.

TAMI-51. HORIZONTAL MITOCHONDRIAL TRANSFER FROM THE TUMOR MICROENVIRONMENT TO GLIOBLASTOMA INCREASES TUMORIGENICITY

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Communication between glioblastoma (GBM) and its microenvironment facilitates tumor growth and therapeutic resistance, and is facilitated through a variety of mechanisms. Organelle transfer between cells was recently observed, including mitochondria transfer from astrocytes to neurons after ischemic stroke. Given the dependence of GBM on microenvironmental interactions, we hypothesized that mitochondria transfer from tumor microenvironment to GBM cells could occur and affect metabolism and tumorigenicity. We interrogated this in vivo by establishing intracranial GBM tumors in mito::mKate2 mice (with trackable fluorescent mitochondria) using syngeneic GFP-expressing tumor cells (SB28 and GL261 models). We also cultured stromal cell types from mito::mKate2 mice with tumor cells, enabling sorting of tumor cells with and without exogenous mitochondria. Confocal microscopy revealed horizontal transfer of mKate2+ mitochondria from mouse cells to implanted GBM cells in vivo and was confirmed by flow cytometry where 20-40% of GBM cells acquired exogenous mitochondria. Transfer was negligible in wildtype mice transplanted with mito::mKate2 bone marrow cells, suggesting that brain-resident cells were the main donors. In vitro, astrocytes and microglia exhibited 5 to 10-fold higher mitochondrial transfer rate than bone-marrow derived macrophages. Seahorse metabolic profiling revealed that GBM cells with mKate2+ mitochondria had 40% lower respiratory reserve compared to cells without exogenous mitochondria. Median survival of mice implanted with SB28 that acquired mitochondria was significantly shorter and in vivo limiting dilution confirmed the frequency of tumor-initiating cells was 3-fold higher in SB28 cells with exogenous mitochondria. Our data indicate that horizontal mitochondrial transfer from brain-resident glia to mouse GBM tumors alters tumor cell metabolism and increases their tumorigenicity. Ongoing studies are assessing gene expression in GBM cells acquiring exogenous mitochondria; validating findings in human specimens; and screening for transfer inhibitor drugs. Horizontal mitochondrial transfer represents a foundational tumor microenvironment interaction contributing to glioblastoma plasticity, and is likely to inform next-generation treatment strategies.

TAMI-52. NEURONAL MECHANISMS OF BRAIN TUMOR INVASION

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Incurable gliomas are characterized by their infiltration into the whole brain. Recently, we described tumor microtubes as a novel structure contributing to glioma cell invasion and uncovered synaptic contacts on glioma cells that drive brain tumour progression. However, the exact effects of neuronal activity on glioma cell motility are yet unclear. Here, we show how a recently described neuronal-like cellular transcription state of glioblastoma cells is correlated to glioma cell invasion *in vivo*. To unravel the details of neuronal features of glioma invasion in space and time, we established a novel approach of intravital imaging for brain tumor cells with a membrane-bound GFP combined with deep learning algorithms that are used to track glioma cell processes with a high temporal resolution over several hours. This approach uncovers how invading tumor microtubes use Levy-like movement patterns indicative of efficient search patterns often employed by animal predators searching for scarce resources such as food. Neuronal activity is able to accelerate the tumor microtube dynamics, accelerate the Levy-like movement patterns and increase the overall invasion speed of glioma cells. These processes are mediated by local calcium transients in glioma cell somata and tumor microtubes. In accordance, genetic manipulation and pharmacological perturbation of AMPA receptors reduces tumor microtube length, number and branching points by interfering with intracellular calcium transients. All in all, the work here uncovers novel neuronal activity-mediated mechanisms of glioma cell invasion, a hallmark of this yet fatal disease.

TAMI-53. CYSTEINE IS A LIMITING FACTOR FOR GLIOMA PROLIFERATION AND SURVIVAL

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BACKGROUND: Little is known about the mechanisms that render cancer cells dependent on certain nutrients from the microenvironment. Cysteine is a non-essential amino acid, since it can be synthetized from methionine through the transsulfuration pathway; moreover, cysteine is also uptake from the diet as cystine. We have investigated the metabolism of cysteine in glioma cell lines, and how cysteine/cystine-deprivation alters their antioxidant response in addition to the effect of this nutrient restriction to viability and proliferation in vitro and in vivo. METHODS: Cysteine metabolism was investigated through LCMS-based 13C-tracing experiments and the expression levels of key enzymes in the transsulfuration pathway were also explored. Finally, a mouse model of IDH1 mutant glioma was subjected to a cysteine/cystine-free diet and tumor metabolism was analyzed by LCMS. RESULTS: Herein, we report the dependence of glioma cells on exogenous cysteine/cystine, despite this amino acid being nonessential. Using several 13C-tracers and analysis of cystathionine synthase and cystathioninase levels, we revealed that glioma cells were not able to upregulate the transulfuration pathway cysteine, which allows methionine to be converted to cysteine in cysteine/cystine deprived conditions. We demonstrated that exogenous cysteine/cystine are crucial for glutathione synthesis, and impact growth and viability. Therefore, we explored the nutritional deprivation in a mouse model of glioma. Animals subjected to a cysteine/cystine-free diet survived longer, with concomitant reductions in glutathione and cysteine plasma levels. At the endpoint higher levels of oxidative stress were detected despite the systemic recovery of cysteine-related metabolites in the plasma. CONCLUSION: The results presented herein reveal an alternative therapeutic approach combining cysteine/cysteine-deprivation diets and treatments involving ROS production by limiting the ability of glioma cells to quench oxidative stress through dietary interventions. Our study highlights a time window where cysteine deprivation can be exploited for additional therapeutic strategies.

TAMI-54. SEXUAL DIMORPHISM IN IRON ACQUISITION IN GLIOBLASTOMA

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Sexual dimorphism in incidence and the clinical outcomes of Glioblastoma (GBM) has been reported, however, our knowledge of contributing biological mechanisms is limited. Iron acquisition is key to robust tumor growth. Upregulation of Transferrin (TF, iron transport protein)/Transferrin receptor (TFR) is critical for found in multiple different cancers, specifically, we have identified H-ferritin (FTH1) as a contributor to iron transport and protection in cancer stem cells. To interrogate brain tumor iron uptake mechanisms,we performed binding studies on homogenized samples of human male and female GBM tissue samples using 125I labeled TF and FTH1. Tumors from males had a- 3.8-fold increased binding of both proteins compared to tumors from females. We interrogated iron uptake in a syngeneic orthotopic mouse model (GL261 cells) using male and female mice. After the tumors were established, radioactive 125I labeled TF and FTH1 proteins were injected retro-orbitally in the mice. After 24 hours, tumors wereremoved, and analyzed for TF and FTH1 uptake. Male tumors showed an increased uptake, of ~ 3.2-fold, as compared to female tumors. There was no significant difference in TF uptake between male and female tumors nor between tumor and matched non-tumor brain tissue. We next queried role of FTH1 in the context of sexual dimorphism in GBM in a FTH1+/- mouse strain developed in our laboratory. Survival was monitored in the mice which were injected with GL261 cells at 3 months. Male mice that had reduced expression of FTH1 had poorer survival as compared to the male wild type controls whereas wild type and FTH+/- females had no major differences in survival outcomes. In summary, this study demonstrates sexual dimorphism in iron acquisition in GBM and animal models further suggesting a pathophysiological role of iron metabolism in GBM development and its possible role in prognosis.

TAMI-56. TARGETING AMINO ACID METABOLIC VULNERABILITIES IN IDH-MUTANT AND IDH-WILDTYPE GLIOMAS

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IDH-wildtype glioma and IDH-mutant glioma have different genetical and metabolic background although their histological appearances are similar. The aim of the study is to reveal the difference in metabolites between IDH-wildtype glioma and IDH-mutant glioma, and to find the ef-