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2013 WFNO-SNO Abstracts

METABOLIC PATHWAYS

MP-001. INVOLVEMENT OF THE KYNURENINE PATHWAY IN HUMAN PRIMARY GLIOMA PATHOPHYSIOLOGY

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The kynurenine pathway (KP) is the principal route of L-Tryptophan (TRP) catabolism leading to the production of kynurenine (KYN), the neuroprotectants, kynurenic acid (KYNA) and picolinic acid (PIC), and the excitotoxic neurotoxin, quinolinic acid (QUIN). The enzymes indoleamine 2,3-dioxygenase-1 (IDO-1), indoleamine 2, 3-dioxygenase-2 (IDO-2) and tryptophan 2,3-dioxygenase (TDO-2) initiate the first step of the KP. Downstream enzymes include kynureninase (KYNU), 3-hydroxyanthranilate 3,4-dioxygenase (3-HAAO), kynurenine hydroxylase (KMO) and 2-amino-3-carboxymuconate semialdehyde decarboxylase (ACMSD). Kynurenine aminotransferase-I (KAT-I) is one of the enzymes responsible for synthesising KYNA. Mounting evidence directly implicates that IDO-1 induction in various tumours is a crucial mechanism facilitating tumour immune evasion and persistence. However, the involvement of the downstream machinery of the KP in brain tumour progression remains unexplored. A complete characterisation of the KP in brain tumours and the role of the KP in maintaining homeostasis between neuroprotection and neurodegeneration in glioma has not yet been investigated. Here we report the first comprehensive characterisation of the KP in cultured human glioma cells and GBM patient plasma. Our qRT-PCR data revealed that interferon-gamma (IFN-γ) (100 IU/ml) stimulation significantly potentiated the expression of IDO-1 IDO-2, KYNU, 3-HAAO, KMO and significantly down-regulated ACMSD and KAT-I expression in cultured human glioma cells. HPLC analysis revealed that IFN-γ stimulation significantly increased KP activity (KYN/TRP ratio), and significantly lowered the KYNA/KYN neuroprotective ratio in human cultured glioma cells. Our HPLC and GCMS data revealed that KP activation was significantly higher and the concentrations of TRP, KYNA, QUIN and PIC and the KYNA/KYN ratio were significantly lower in GBM patient plasma ($n = 18$) compared to controls. These results provide further evidence for the involvement of the KP in glioma pathophysiology and highlights a potential role of KP products as novel and highly attractive therapeutic targets to evaluate for the treatment of brain tumours.

MP-002. A HEXOKINASE 2 (HK2) ASSOCIATED GENE SIGNATURE IDENTIFIES THE AZOLE CLASS OF ANTIFUNGALS AS EFFECTIVE DRUG COMPOUNDS TARGETING TUMOUR METABOLISM IN GLIOBLASTOMA

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Rapidly proliferating tumour cells preferentially use aerobic glycolysis over oxidative phosphorylation (OXPHOS) to support growth and survive unfavorable microenvironment conditions. This metabolic reprogramming is referred to as the "Warburg effect" and offers a novel way to target cancer cells. We previously demonstrated that the glycolytic enzyme hexokinase 2 (HK2) is crucial for the Warburg effect in human glioblastoma multiforme (GBM), the most common malignant brain tumor. Furthermore, HK2 has little to no expression in normal brain making it an attractive target for targeting the Warburg effect. However, no direct inhibitor of HK2 exists so we explored whether a system biology approach to identify gene networks regulated by or associated with HK2 that could lead to promising treatment strategies. Using HK2 knockdown by siRNA in established GBM cell lines and primary GBM cultures we established gene signatures and networks associated with HK2 expression, identifying 1000 genes with a 2 fold change with p-value <0.01 and false discovery rate of $<1\%$. Loss of HK2 led to attenuation of HIF1a, mTOR, PI3K and AKT signaling pathways affecting tumour cell functions including invasion, glucose metabolism and proliferation. Using a small drug screen of 30 compounds that were predicted to repress HK2 expression and associated metabolic gene signatures we identified the azole class of antifungals as inhibitors of tumour metabolism by reducing proliferation, lactate production, glucose uptake and increasing O2 consumption in GBM cells but not primary normal human astrocytes or normal neural stem cells. Current work is focused on the in vivo efficacy of these azole compounds in pre-clinical orthotopic xenograft mouse models and transgenic models of GBM. Additionally, novel metabolic mechanisms by which these antifungals work are currently being elucidated. In summary, the azole class of antifungals may represent a new way of targeting tumour metabolism in tumours dependent on HK2.

MP-003. LDHA SILENCING IN IDH MUTANT GLIOMAS

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Mutations of IDH1/2 were initially thought to confer survival and proliferation advantages by promoting the Warburg effect. However, a recent report clarifies that 2-hydroxyglutarate (2-HG), product of the IDH mutant enzyme, activates the prolyl-hydroxylase leading to HIF1α degradation in glioma cells. The subsequent down-regulation of HIF1α target genes could have important consequences, including inhibition of the Warburg glycolytic shift in IDH mutant (IDHmt) glioma cells. Here, we show that HIF1α responsive genes, including several that are essential for glycolysis, are under-expressed in IDHmt gliomas and derived brain tumor stem cells (BTSCs). To this finding, we add that HIF1α responsive genes involved in glycolysis may be down-regulated in BTSCs derived from IDHmt gliomas, whether they retain the mutant IDH allele, or not, and whether or not they produce 2-HG. Focusing further on the key glycolytic enzyme lactate dehydrogenase-A (LDHA), we demonstrate its silencing in IDHmt derived BTSCs, including those that have lost the mutant allele (mIDHwt), matched BTSC xenografts and their parental glioma tissues. Interestingly, in IDHmt/mIDHwt BTSC lines and IDHmt gliomas tissue samples, the LDHA promoter is hypermethylated. Moreover, expression of mutant IDH1 in immortalized human astrocytes (NHAs) is sufficient to increase LDHA promoter methylation, demonstrating a direct link between IDH mutation and LDHA promoter hypermethylation. Together, these findings demonstrate IDHmt dependent silencing of LDHA via promoter hypermethylation. We thus propose that silencing of LDHA via promoter hypermethylation is a result of the IDHmt-dependent epigenetic remodelling and can persist even after the "loss" of the mutant IDH1 allele and the cessation of 2-HG production. The silencing of LDHA and down-regulation of other glycolytic essential genes, while surprising, raise the intriguing possibility that IDHmt gliomas may have limited capacity for glycolysis. This unique characteristic could in turn contribute to their slow growth and better prognosis compared to IDHwt gliomas.

MP-004. METABOLIC DISRUPTION AS A MECHANISM TO POTENTIATE TEMOZOLOMIDE THERAPY IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is an incurable form of brain cancer with an average life expectancy of approximately one year. Despite surgical

resection and adjuvant therapy, mortality occurs in close to 100% of the patients, presumably due to inherent drug and radio-resistance. Temozolomide (TMZ) has become the standard of care for GBM. TMZ induces DNA damage via methylation of DNA at the O6 and N7 positions of guanine, which results in cell cycle checkpoint arrest and apoptotic death. However, O6-methylguanine-DNA-methyltransferase (MGMT) can repair these damages unless methylation of the promoter for the enzyme prevents its transcription. TMZ resistance can also result from the process of cellular autophagy whereby a cell utilizes its own organelles as an alternate energy source. Autophagy has been shown to provide organellar lipids for fatty acid oxidation (FAO) and we have previously demonstrated that FAO in tumor cells protects cells from apoptosis. Mitochondrial uncoupling proteins serve to lower mitochondrial membrane potential and promote FAO. Furthermore, the activity of UCP2 to depolarize the mitochondrial membrane may prevent free radical damage. We hypothesized that etomoxir, a clinically approved inhibitor of carnitine palmitoyl transferase, will suppress FAO, inhibit the activity of UCP2, and promote Fas:FasL-mediated cell death by increasing DNA damage in glioma cells. Here we show that both C6 rat glioma and U263 human glioma cell lines express UCP2, exhibit low mitochondrial membrane potential, and are resistant to many apoptosis-inducing stimuli. We demonstrate that when treated with etomoxir, the cells have increased mitochondrial membrane potential and increased DNA damage. In addition, when combined with TMZ, these genotoxic activities appear to act synergistically to induce even greater DNA damage, concomitant expression of Fas and Fas ligand, and when combined, a significantly increased percentage of cell death.

MP-005. META-ANALYSIS OF CANCER CELL LINE GENOMES BASED ON THEIR RESPONSE TO ALTERNATING ELECTRIC FIELDS (TTFIELDS)

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Tumor Treating Fields (TTFields) are an established anti-mitotic treatment modality approved by the FDA for the treatment of recurrent GBM. The inhibitory effect of TTFields was demonstrated in numerous cancer cell lines with some variability in the response of different cell lines. The goal of the present study is to compare characteristics of cell lines based on their response pattern to TTFields. Sixteen different human cancerous cell lines were treated using TTFields, each at its specific optimal frequency with the same nominal field intensity (1.8 V/cm). Cell survival, cell volume and clongenicity were determined. Functional analysis of differentially expressed genes and mutations associated with response to TTFields was carried based on the Cancer Cell Line Encyclopedia (CCLE) database. Functional categories were ranked using DAVID Bioinformatics Resources. The inhibitory effect of TTFields was found to be normally distributed around an average of 55% with minimal and maximal inhibitory effects of 15% and 66%, respectively. Cell volume increase of 33% to 116% was evident in 15 out of the 16 cell lines tested. Reduced clongenicity in over 90% of cell lines treated with TTFields was demonstrated with an average of 55 + 34% of control. Functional analysis of cell line gene expression and mutation data revealed enriched pathways related to DNA replication and DNA damage repair response. This work is the first multi parameter, large scale comparison of cancerous cell line response to TTFields. Results demonstrate that in addition to their known direct anti-mitotic effects, TTFields exhibit long term inhibitory effects on treated cell clongenic properties, possibly through the DNA replication and DNA damage repair response pathways.

MP-006. MECHANISTIC INSIGHT INTO "GO OR GROW" BEHAVIOR OF GLIOMA CELLS: microRNA-451/AMPK NEGATIVE FEEDBACK LOOP

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Glioblastoma multiforme (GBM), the most common and aggressive primary brain tumor is characterized by the hallmarks of cellular heterogeneity, rapid proliferation and extensive invasion. Glioma cells within rapidly growing tumor must adapt to frequent fluctuations in nutrients supply. In rich environment glioma cells rapidly proliferate but do not migrate to significant extent. Under conditions of nutrient deprivation glioma cells engage in more migratory behavior while slowing down their growth. We describe rapid and robust, glucose-regulated feedback mechanism mediated by migration inhibitor microRNA-451 and growth inhibitor AMPK kinase, allowing cells to switch between those two states. In low glucose conditions, AMPK is activated by conformational change and phosphorylation by LKB1 kinase complex, leading to growth inhibition. Active AMPK phosphorylates transcription factor OCT1, rendering it unable to bind DNA in proximity to miR-451 locus ensuing blockade of its expression and thus allowing enhanced migration. In abundant glucose, AMPK is inactivated allowing unrestricted growth. Inactive AMPK allows OCT1 to bind miR-451 promoter inducing its high expression and leading to blockade of migration and ensuing inhibition of AMPK by targeting LKB1 kinase co-factor. We demonstrated that forced over-expression of miR-451 disrupts mechanism described above, leading to impaired cellular survival under stress such as glucose deprivation or anticancer therapy. Thus, in light of our results, we conclude that miR-451 possesses unique ability to inhibit glioma cells invasiveness, while sensitizing them to conventional therapy.

MP-007. HYPOXIA AND OXYGENATION INDUCE A METABOLIC SWITCH BETWEEN THE PENTOSE PHOSPHATE PATHWAY AND GLYCOLYSIS IN GLIOMA STEM-LIKE CELLS

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Hypoxia prevents differentiation of normal and tumor stem cells. Glioblastomas are hypoxic tumors, but oxygen levels fluctuate, requiring metabolic flexibility for progression. We compared effects of acute versus chronic hypoxia and oxygenation, using pairs of glioblastoma stem-like cell lines established from 4 tumors under chronic normoxia (21% O2) or hypoxia (1% O2) and subsequently exposed to acute hypoxia or oxygenation, respectively. Gene expression profiling showed that chronic hypoxia predominantly activated neural development pathways, whereas acute hypoxia upregulated metabolic pathways. Bioinformatic analyses (confirmed by immunoblot and qPCR analyses) revealed an inverse regulation between the pentose phosphate pathway (PPP) and glycolysis: expression of PPP enzymes was upregulated by acute oxygenation but downregulated by hypoxia, whereas glycolysis enzymes, particularly those of the preparatory phase, were regulated inversely. Fluxomic analysis demonstrated reduced glucose flux through the PPP under hypoxia in favor of flux through glycolysis. PPP enzyme expression was elevated in human glioblastomas compared to normal brain as indicated by Rembrandt database and tissue microarray analyses, especially in highly proliferative tumor regions, whereas expression of parallel preparatory phase glycolysis enzymes was reduced in glioblastomas, except for strong upregulation in severely hypoxic regions (pseudopalisades). In vitro, acute and chronic hypoxia consistently enhanced GS cell migration but reduced proliferation, whereas oxygenation had opposite effects. shRNA knockdown of glucose-6-phosphate dehydrogenase or aldolase C, the most strongly inversely regulated PPP and glycolysis enzymes, respectively, as well as enzyme inhibitor studies indicated that inhibition of glycolysis decreased migration but increased proliferation, whereas PPP inhibition had opposite effects. Our findings extend Warburgs observation that tumor cells predominantly utilize glycolysis for energy production, by suggesting that the PPP, which supplies pentoses and NADPH for RNA/DNA and fatty acid synthesis, is elevated in rapidly proliferating tumor cells but suppressed by acute severe hypoxic stress, favoring glycolysis to protect cells against hypoxic damage.

MP-008. EVALUATION OF FATTY ACID SYNTHASE EXPRESSION AS PREDICTIVE FACTOR FOR AGGRESSIVENESS IN MENINGIOMAS

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BACKGROUND AND PURPOSES: Based on clinical and pathologic findings, most meningiomas are benign although predicting the behavior of individual meningiomas remains difficult. Even grade I meningiomas can manifest clinically aggressive behaviors such as penetration of the arachnoidal border and destruction of the bone. Residual tumors may regrow rapidly and totally resected tumors can recur. Re-programming of metabolic pathways including glycolysis, lipogenesis and nucleotide synthesis is a hallmark of physiological changes in cancer cells. Fatty acid synthase (FAS), the enzyme responsible for de novo synthesis of fatty acids, has emerged as a potential therapeutic target for several cancers. In this study, we investigated the role of FAS in meningiomas. MATERIALS AND METHODS: Paraffin-embedded

samples (57 grade I, 27 grade II and III, 8 radiation-induced tumors) were used for immunohistochemical study of FAS. FAS expression was graded semi quantitatively as follows; no (−), vague (±), weak patchy (+), focal strong (++) and over 50% expression (+++). We defined one plus to three plus as positive expressions. RESULTS: While its expression was increased in grade II and III meningiomas (62.9%) compared with grade I tumors (29.8%) (Chi-square test: $p < 0.001$), FAS was expressed in grade I tumors with high MIB-1 index and infiltration into surrounded tissues. All radiation-induced meningiomas expressed FAS and its expression was positively correlated with the MIB-1 index ($p < 0.005$). CONCLUSIONS: Our findings suggest that increased FAS expression reflects the aggressiveness of meningiomas and that it may represent a novel therapeutic target for the treatment of unresectable or malignant tumors.

MP-009. ANATOMICAL DISTRIBUTION OF ONCOMETABOLITES IN IDH1 MUTANT GLIOMAS REVEALED BY *IN SITU* HIGH RESOLUTION IMAGING MASS SPECTROMETRY

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The dramatic increase in D-2-hydroxyglutarate (D2-HG) in glial tumors presenting a heterozygous mutation in isocitrate dehydrogenase (IDH) is a key indicator of aberrant metabolic circuitry in these tumors. IDH1 is a dimeric enzyme that converts isocitrate to α-ketoglutarate (α-KG). In IDH1-mutant tumors, the enzyme forms wildtype-mutant heterodimers and α-KG is further processed to D2-HG. D2-HG is proposed to act as an oncometabolite and to induce hypermethylation by inhibiting the activity of α-KG-dependent methylases. However the precise oncogenic mechanism and metabolic alterations resulting from the mutant phenotype are poorly understood. Since glioma cells carrying the IDH mutation are notoriously difficult to grow in vitro, most current studies addressing the role of D2-HG overexpress mutant IDH1 in cell lines on a wildtype background. This however strongly disrupts the stoichiometry of the mutant versus wildtype subunits, which appears to be crucial for the function of the oncogenic enzyme. We recently reported on the generation of IDH1 mutant xenografts derived from human oligodendroglial tumors with 1p19q deletion. Using in situ mass spectrometric imaging on tumor sections we found that the high levels of D2-HG are specifically localized in the tumor bed but not in the surrounding brain parenchyma. Interestingly, α-KG levels were not significantly different from IDH1-wildtype tumors and normal brain, suggesting that the α-KG pool is maintained in IDH1-mutant tumors despite its strong conversion to D2-HG. We now further applied high resolution mass spectrometry (LESA and MALDI imaging) to describe the metabolic profiles of IDH-mutant gliomas in situ on xenograft tissue sections. In addition to D2-HG, we find a subset of unexpected metabolites with a highly differential pattern in IDH1-mutant tumors and we provide an anatomical distribution of these metabolites in situ. This emerging approach provides unprecedented information on intratumoral heterogeneity at the metabolic level and novel mechanistic insight into IDH1 mutant gliomas.

MP-011. THE ENDOGENOUS TRYPTOPHAN METABOLITE AND NAD+ PRECURSOR QUINOLINIC ACID CONFERS RESISTANCE OF GLIOMAS TO OXIDATIVE STRESS

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Quinolinic acid (QA) is a product of tryptophan degradation and may serve as a precursor for nicotinamide adenine dinucleotide (NAD+), an important enzymatic cofactor for enzymes such as the DNA repair protein poly (adenosine diphsophate[ADP]-ribose)polymerase. Pathologic accumulation of QA has been found in neurodegenerative disorders including Alzheimer's and Huntington's disease, where it is thought to be toxic for neurons by activating the NMDA receptor and inducing excitotoxicity. While many tumors including gliomas constitutively catabolize tryptophan, it is unclear whether QA is produced in gliomas and whether it is involved in tumor progression. Here we show that QA accumulated in human gliomas and was associated with a malignant phenotype. QA was produced by microglial cells as expression of the QA-producing enzyme 3-hydroxyanthranilate oxygenase (HAAO) was confined to microglia in glioma tissue. Human malignant glioma cells but not non-neoplastic astrocytes expressed quinolinic acid phosphoribosyltransferase (QPRT) to utilize QA for NAD+ synthesis and prevent apoptosis when de novo NAD+ synthesis was blocked. Oxidative stress, temozolomide and irradiation induced QPRT in glioma cells. QPRT expression increased with malignancy. In recurrent glioblastomas after radiochemotherapy, QPRT expressionwas associated with a poor prognosis in two independent datasets. Our data indicate that neoplastic transformation in astrocytes is associated with a QPRT-mediated switch in NAD+ metabolism by exploiting microglia-derived QA as an alternative source of replenishing intracellular NAD+ pools. The elevated levels of QPRT expression increases resistance to oxidative stress induced by radiochemotherapy, conferring a poorer prognosis. These findings have implications for therapeutic approaches inducing intracellular NAD+ depletion such as alkylating agents or direct NAD+ synthesis inhibitors and identify QPRT as a potential therapeutic target in malignant gliomas.

MP-012. GLIOBLASTOMA STEM CELLS REQUIRE IRON FOR MAINTAINING TUMORIGENIC POTENTIAL

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Within the Glioblastoma (GBM) tumor bulk and microenvironment, iron levels are elevated, presumably involved in angiogenesis and tumor growth. While interrogating the proteins necessary for iron utilization have provided tremendous insight into iron regulation in central nervous system pathologies as well as other cancers, the role of iron in GBM and the potential therapeutic value of regulating tumoral iron remains unclear. Previously, we have shown that glial progenitors accumulate iron, which was associated with increased migration and proliferation. Based on these data and the apparent role for iron in promoting angiogenesis and protecting tumor cells from DNA damage, I hypothesized that in GBM, iron is essential for neoplastic metabolism, regulating tumor cell growth promoting GBM-mediated angiogenesis. Iron is upregulated in GBM specimens compared to other brain tumors and non-tumor specimens with increased metabolism associated with poor survival outcome among glioma patients. Within the GBM cellular hierarchy, iron regulatory proteins are differentially expressed, being notably elevated in cells that have stem-like properties; referred to as GBM stem cells (GSCs). The significance of GSCs has been supported by studies from our group and others that these cells are resistant to conventional therapy. Along with recent findings linking elevated iron usage to chemoresistance, these studies suggest the importance of identifying mechanisms, potentially involving iron use which regulate GSC maintenance. In addition to their chemoresistance properties, GSCs promote angiogenesis which involves stimulation and upregulation of hypoxia-inducible factors (HIFs). Not only is iron present in human GBM xenografts but uptake is induced in GSCs exposed to hypoxia and/or HIFs. Disrupting iron metabolism in GSCs using shRNA targeted against iron proteins reduces cell growth and decreases their ability to form tumorspheres as well as establish tumors in vivo. These results suggest that iron is important for GSC maintenance and tumorigenic potential.

MP-013. REGULATING TUMOR METABOLISM AND RESPONSE TO THERAPY WITH HEXOKINASE II (HKII) INHIBITION IN GLIOBLASTOMA (GBM)

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BACKGROUND: Refocusing research on altered tumor metabolism has lead to emergence of therapeutic concepts aimed at inhibiting cancer-specific metabolic programs or drivers. Glioblastoma (GBM), similar to many other cancers, exhibits enhanced aerobic glycolysis with concomitant lactate production, a phenomenon known as the Warburg effect. We have demonstrated that preferential expression of Hexokinase II (HKII) is a critical mediator of metabolic reprograming in GBMs and its selective inhibiting is a potential therapeutic strategy for sensitization of GBM tumors to radiation (RAD) and/or temozolomide (TMZ). EXPERIMENTAL DESIGN: We generated conditional knockdown of HKII in human GBM cell lines (U87 and GSC), established

intracranial tumor xenografts in mice and assessed anti-cancer effects of HKII knockdown in combination with TMZ and/or RAD in vivo using bioluminescent imaging and histopathology. RESULTS: Conditional inhibition of HKII disrupts energy homeostasis, reduces cell viability, colonogenic potential and induces DNA damage and radio-chemo sensitization under hypoxic conditions. In vivo results confirmed that conditional loss of HKII results in a significant survival benefit in GBMs models ($p < 0.0001$). Similarly, we demonstrated for the first time that HK2 knockdown sensitizes GBM tumors to concomitant TMZ and RAD and significantly prolongs the median survival of the mice ($p = 0.0003$). Loss of HKII resulted in GBM remodeling such that HK2 knockdowns show increased necrosis, hypoxic fraction, infiltration of inflammatory cells and reduced vascularization. CONCLUSION: The significant synergistic effect upon combining HK2 knockdown with RAD/TMZ suggests targeting the Warburg effect through HK2 inhibition is a promising therapeutic strategy for improving the efficacy of current therapeutic regimens in GBMs.