ABSTRACTS

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S1 – Session1 0930-0945

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Identification of the azole class of antifungals as potent inhibitors of glioblastoma growth and tumour metabolism

(Young Investigator Award Winner - Basic/Translational Science)

S. Agnihotri, K. Burrell, S.K. Singh, A.Vartanian, K. Aldape, G. Zadeh

Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto, Ontario

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. 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 over 1000 genes with a 2 fold change with p-value <0.01. Loss of HK2 led to attenuation of several pro GBM signaling pathways affecting tumour cell invasion, glucose metabolism and proliferation. Using a small drug screen we identified the azole class of antifungals as inhibitors of tumour metabolism by reducing proliferation, lactate production, glucose uptake in GBM cells but not primary normal human astrocytes or normal neural stem cells. Interestingly, several antifungal Azole compounds were more potent at killing GBM cells in hypoxic conditions. 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. In summary, the azole class of antifungals may represent a new way of targeting tumour metabolism in tumours dependent on aerobic glycolysis.

S2 – Session1 0945-1000

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CIC in neurodevelopment and oligodendroglioma

AD Rogers[1], R Dixit[2], SO Lawn[2], S Li[3], CJ Schuurmans[3], JG Cairncross[1], JA Chan[2]

Departments of Clinical Neuroscience[1], Pathology & Laboratory Medicine[2] and Biochemistry & Molecular Biology[3], University of Calgary, Calgary, Alberta

Oligodendrogliomas (ODG) are distinctive brain tumours composed of cells resembling oligodendrocyte precursor cells (OPCs). Genetic hallmarks of ODGs include 1p/19q chromosomal co-deletion and IDH1/2 mutation. Recently, the gene encoding Capicua (CIC), on chr19q13.2, was identified as mutated in most ODGs with concurrent 1p/19q loss and IDH1/2 mutation a genetic signature rare in other cancers. Mutation of the retained 19q CIC allele is likely functionally important, but how it contributes to ODG biology is unknown. The aims of this study are to characterize the temporal and spatial expression of Cic in the normal mouse cerebrum, and to determine if CIC loss affects proliferation or differentiation of neural progenitors. To examine CIC expression, immunofluorescence staining was performed on forebrain tissue over a developmental timecourse. CIC biologic functions were determined using a loss-of-function approach, introducing CIC shRNA or control shRNA into neural progenitors. Cells were examined for proliferation, and for cell identity using a panel of markers. Our data supports a role for Cic in regulating several processes in neural progenitors that are relevant to cancer including proliferation and, possibly, differentiation. CIC loss due to mutational inactivation may thus deregulate processes relevant to oligodendrogliomagenesis.

S3 - Session1 1000-1015

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Targeting Glioblastoma Invasion with GSK-3 inhibitors: Rapid Effects on the EMT Marker Vimentin

Michal O. Nowicki[1], E. Antonio Chiocca[1], Sean E. Lawler

Harvey Cushing Neuro-oncology Laboratories, Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

Tumor cell invasion of surrounding normal brain remains a formidable obstacle to effective glioblastoma therapy. We previously identified that inhibitors of the protein kinase GSK-3

are able to specifically block glioblastoma invasion in vitro and in vivo, where they also prolong survival in glioblastoma animal models. To better understand the functions of GSK-3 in glioblastoma we used proteomics which revealed major changes in cytoskeletal proteins, with downregulation of the EMT marker vimentin being the most significant alteration. Vimentin is an intermediate filament protein that functions as an organizer of a number of critical proteins involved in attachment, migration, and cell signaling. The downregulation of vimentin was rapid and due to alterations in its dynamics in response to GSK-3 inhibition. GSK-3 and vimentin were shown to associate with each other in glioblastoma cells, and reduction in vimentin phosphorylation was observed suggesting it may be a novel substrate of GSK-3. We showed that vimentin is highly expressed in patient glioblastoma samples and higher levels of vimentin are associated with poorer prognosis. Vimentin knockdown also reduced glioblastoma cell migration. The mechanism of action of GSK-3 inhibition in the context of glioblastoma invasion and the potential of developing a therapeutic strategy based on these observations will be discussed.

S4 - Session1 1015-1030

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Bone marrow derived immune cells and their role in tumor heterogeneity

K. Burrell, S. Singh, S. Agnihotri, R. Hill, G. Zadeh

Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto, Ontario

The role of bone marrow derived cells (BMDC) in tumor neo-vascularization remains controversial. We previously demonstrated recruitment and migration of distinct subpopulations of BMDCs to Glioblastoma Multiforme (GBM), and association with the GBM vasculature in a highly tumor region dependent manner. Continuation of this work has focused on establishing the molecular alterations generated in BMDC as a consequence of interaction with the GBM tumor microenvironment. Our second goal has been to establish whether the tumor microenvironment influences differentiation and contribution of BMDC in GBMs. Intracranial xenograft models were created in chimeric mice generated by reconstituting the bone marrow with fluorescent (gfp or dsred) BM. BMDC were isolated from the GBM microenvironment using FACS sorting of the fluorescent tag at early and late stages of GBM growth, in addition to following treatment with RTx and AATx. We demonstrate that VEGF inhibition though indirect mechanisms, RTx, and direct mechanisms, VEGFTRap, can alter the differential recruitment of pBMDCs observed through normal tumor progression. It is known that inhibition of VEGF leads to an increase in ANG2 signal, which may in turn be linked to the recruitment of pBMDCs. Through addition of an ANG2 inhibitor we can show that through concomitant VEGF and ANG2 inhibition, pBMDC recruitment can be prevented. These results suggest that BMDC contribute through distinct mechanisms to tumor invasion and neo-vascularization and thereby targeting the specific cascade of angiogenic and invasion factors will prevent the pro-tumoral contribution of BMDC in supporting tumor growth and aiding in response to therapy.

S5 – Session1 1100-1115

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Role of hexokinase 2 (HK2) in modulating tumor metabolism and response to therapy in glioblastoma

A. Vartanian, K. Burrell, S.K. Singh, S. Jalali, S. Agnihotri, G. Zadeh

Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto, Ontario

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 2 (HK2) is a critical mediator of metabolic reprograming in GBMs and its inhibition is a potential therapeutic strategy for sensitization of GBM tumors to radiation (RAD) and/or temozolomide (TMZ**).** Our results indicate that conditional HK2 inhibition disrupts energy homeostasis and sensitizes GBMs to radiation and chemotherapy. In GBM xenografts, conditional HK2 loss sensitizes GBM tumors to concomitant RAD/TMZ and results in a significant survival benefit in the mice. Moreover, loss of HK2 resulted in GBM remodeling with HK2 knockdowns showing increased necrosis, hypoxia, inflammatory infiltration and reduced vascularization. We demonstrate that targeting a key metabolic enzyme involved in the Warburg effect might improve the efficacy of current therapeutic regimen and provide a unique paradigm for the management of GBMs.

S6 – Session1 1115-1130

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Classifying medulloblastoma into molecular subgroups: Means, motive, and opportunity

D.J.H. Shih[1], P.A. Northcott[2], M. Remke[1], A. Korshunov[2], D.T.W. Jones[2], M. Kool[2], S.M. Pfister[2], M.D. Taylor[1]

[1]Hospital for Sick Children, Toronto, Ontario; [2]German Cancer Research Center, Heidelberg, Germany

Current medulloblastoma protocols stratify patients based on clinical features: patient age, metastatic stage, extent of resection, and histological variant. Stark prognostic and genetic differences between the four subgroups suggest that subgroup-specific molecular biomarkers could improve patient prognostication. Method: Molecular biomarkers were identified from a discovery set of 673 medulloblastomas from 43 cities around the globe.