circumvents cisplatin-resistance and is less dependent on p53 activity than cisplatin, these results suggest a molecular mechanism for VAL-083 that differs from both TMZ, BCNU and cisplatin. They further suggest that irreparable DNA damage induced by VAL-083 is impervious to common strategies employed by cancer cells to escape effects of alkylating drugs used in GBM treatment.

PS2 – 167

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CIC Deficiency is Associated with Dysregulation of Genes Involved in Cell Adhesion and Developmental Processes

V.G. LeBlanc[1], S. Chittaranjan[1], M. Firme[1], S.Y. Chan[1], J. Song[1], A. Lee[1], S. Yip[2], M.A. Marra[1,3]
[1]BC Cancer Agency Genome Sciences Centre
[2]Departments of Pathology & Laboratory Medicine
[3]Medical Genetics, University of British Columbia, Vancouver, BC
vleblanc@bcgsc.ca

Somatic mutations in the Capicua (*CIC*) gene were first identified in Type I low-grade gliomas (LGGs), which are characterized by 1p/19q co-deletions and *IDH* mutations. They are found at frequencies of ~50-70% in this glioma subtype, and have since been identified in ~40% of stomach adenocarcinomas (STADs) of the microsatellite instability (MSI) subtype; however, the role of these somatic mutations in malignancy has yet to be established. In *Drosophila*, CIC functions as a transcriptional repressor whose activity is inhibited upon activation of the mitogen-activated protein kinase (MAPK) signalling pathway. Though mammalian CIC appears to retain these functions, only three of its target genes have been established in human cells: *ETV1*, *ETV4*, and *ETV5* (*ETV1/4/5*). To further probe CIC's transcriptional network, we developed *CIC* knockout cell lines and performed transcriptomic and proteiomic analyses in these and in control cell lines expressing wild type *CIC*, identifying a total of 582 differentially expressed genes. We also used RNA-seq data from The Cancer Genome Atlas (TCGA) for Type I LGGs and STADs to perform additional differential expression analyses between CIC-deficient and CIC-expressing samples. Though gene-level overlap was limited between the three contexts, we found that CIC appears to regulate the expression of genes involved in cell-cell adhesion, metabolism, and developmental processes in all three contexts. These results shed light on the pathological role of *CIC* mutations and may help explain why these have been associated with poorer outcome within Type I LGGs.

PS2 – 171

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Bmi1 Identifies Treatment-Refractory Stem Cells in Human Glioblastoma

P. Vora[1], M. Qazi, C. Venugopal, M. Subapanditha, S. Mahendram, C. Chokshi, M. Singh, D. Bakhshinyan, N. McFarlane, S. Singh
[1]McMaster University, Hamilton, ON
pavora@mcmaster.ca

Glioblastoma (GBM) is an aggressive brain tumor that is resistant to conventional radiation and cytotoxic chemotherapies. We hypothesize that brain tumor initiating cells (BTICs), a subpopulation of treatment-resistant cells with stem cell properties cause tumor relapse and a subset of neural stem cell genes regulate BTIC self-renewal, driving GBM recurrence. We adapted the existing treatment protocol for adults with primary GBM for in vivo treatment of immunocompromised mice engrafted with GBMs. Post-chemoradiotherapy, the recovered GFP+ GBMs were profiled for self-renewal and expression of critical stem cell genes. Using invitro and invivo gain-of-function/loss-of-function experiments, we investigated the regulatory functions of Bmi1 in primary neural stem & progenitor cells (NSPCs) and GBM tumor formation. Finally, global RNA-Seq profiling was performed to understand the consequences of Bmi1 dysregulation on target gene expression. GBM cells showed an increase in Bmi1 levels post-chemoradiotherapy, suggesting the presence of a treatment-refractory BTICs. GFP+ cells extracted from treated xenografts had higher self-renewal and BTIC marker expression. Although treated mice responded to therapy, we observed tumor relapse with increased Bmi1 expression. Knockdown of Bmi1 diminished self-renewal and proliferation of GBM cells and delayed tumorigenesis, highlighting a critical role for Bmi1 in tumor maintenance. Conversely, over-expressing Bmi1 in NSPCs failed to initiate tumor formation in vivo. Using high-throughput sequencing data, we generated a map of signaling pathways dysregulated in GBM that may lead to tumor recurrence. Our data confirms the existence of a rare treatment-refractory BTIC population with enhanced self-renewal capacity that escapes therapy and drives tumor relapse.

PS2 – 172

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Preclinical Validation of a Novel CD33/CD3 Bispecific T-Cell Engager (BiTE) Antibody to Target Patient-Derived Glioblastoma Cells

P. Vora[1], C. Venugopal, C. Choksi, M. Qazi, J. Adams, M. London, M. Subapanditha, M. Singh, D. Bakshinyan, S. Sidhu, J. Moffat, S. Singh
[1]McMaster University, Hamilton, ON
pavora@mcmaster.ca

Glioblastoma (GBM), an aggressive primary adult brain tumor, is feared for its near uniformly fatal prognosis. Despite the use of aggressive treatment including surgical resection, radiotherapy and chemotherapy, the outcome of patients with GBM has failed to improve significantly. Numerous studies have implicated CD133+ GBM subpopulation as driver of chemo- and radio-resistance. CD133 expression correlates with disease progression, recurrence, and poor overall survival of GBM patients. Here, we describe the preclinical evaluation of a recombinant CD133xCD3 bispecific T-cell engager (BiTE) antibody that redirects human polyclonal T cells to CD133+ GBM cells, inducing very potent anti-tumor response. CD133-specific BiTE was constructed; with one arm recognizing the tumor antigen (CD133) while the second is specific to CD3 antigen. Using CD133high and CD133low primary GBM lines, we validated the binding of BiTEs to CD133+ GBMs and CD3+ T cells. In order to test the ability of BiTEs to functionally elicit CD133-specific cytotoxic responses in vitro, we performed killing assays. We observed CD133-specific BiTE mediated T cell activation and redirection to kill CD133-expressing GBM cells in a co-culture of T cells and GBM cells. The killing was more efficient in CD133high GBMs compared to CD133low GBMs, validating its specificity to target CD133+ BTICs. Treatment with BiTEs yielded significant reductions in

brain tumor burden in vivo. These data offers compelling evidence that BiTE-mediated cytotoxicity against treatment-resistant CD133+ GBMs could provide a very potent, specific and can be a novel therapeutic strategy for GBM patients.

PS2 – 174

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Hexokinase 2 Drives Radio-Resistance through ERK Signaling and Sensitizes Cells to Azole Compounds

S. Agnihotri[1], M. Li, M.R. Wilson, K. Burrell, A. Alamsahebpour, K. Aldape, G. Zadeh
[1]MacFeeters Hamilton Center for Neuro-Oncology, Princess Margaret Cancer Center, Toronto, ON
sameer.agnihotri@gmail.com

Our ongoing work has demonstrated that hexokinase 2 (HK2) but not HK1 or HK3 is a critical mediator of tumour glycolysis and mitochondrial metabolism in Glioblastoma (GB). Furthermore, HK2 is highly expressed in GB but not in normal brain making it an attractive therapeutic target. Our current findings now support that loss of HK2 alters tumor vasculature, increases sensitivity to radiation, and confers a significant survival benefit in several GB xenograft-bearing mice. Using a genome wide transcript analysis, we identified that loss of HK2 attenuates several pro-growth signaling pathways in GB including ERK signaling. Mechanistically, ERK rescue experiments in HK2 depleted cells rescues cell sensitivity to radiation and reduces DNA damage. Furthermore using a systems biology approach and a rationale drug screen we identified several antifungal agents in the azole class as to inhibit tumor metabolism and growth in HK2 expressing GB cells. Loss of HK2 in GB cells dampened the effect of several azoles suggesting that the mechanism of action is mediated in part through HK2. Furthermore, we tested several azole compounds known to cross the blood brain barrier in vivo. Clinically achievable doses of azoles as single agents increased survival in several orthotopic xenograft GB mouse models. In summary, HK2 drives several oncogenic pathways associated with GB including ERK signaling and sensitizes tumour cells to the azole class of antifungals. Future work will determine whether azoles work synergistically with radiation and temozolomide and elucidate the mechanisms by which they inhibit GB growth in HK2 expressing cells.

PS2 – 178

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Brevican-Specific Peptides for the Development of Next-Generation Targeted Theranostics for High Grade Gliomas

C.F. Cho[1], K. Kasai, J. Wolfe, K. Hornburg, F. Bononi, L. Luyt, B. Pentelute, M. Viapiano, E.A. Chiocca, S. Lawler
[1]Harvard Medical School, Cambridge, Massachusetts
ccho@bwh.harvard.edu

High-grade gliomas are deadly cancers, and current standard-of-care has demonstrated limited success. The ability to specifically target glioma cells can allow for the development of improved theranostic agents leading to better detection methods, as well as safer anti-cancer therapies. Brevican (Bcan), a CNS-specific protein is upregulated in glioma cells and correlates with tumor progression. Particularly, a Bcan isoform lacking normal glycosylation, called B/bDg is a unique glioma marker and is not expressed in non-cancerous tissues. Therefore, B/bDg represents a valuable target for anti-cancer strategies. We describe here the discovery of novel high-affinity B/bDg-targeted peptides using rapid combinatorial library screening approaches and a microfluidic sorting device of our own design. Briefly, a one-bead-one-compound (OBOC) peptide library was screened against small magnetic particles decorated with B/bDg. Positive "hit" beads labeled with magnetic particles were isolated using an inexpensive but yet, accurate and high-throughput in-house microfluidic magnetic-activated sorter. These hits were exposed to cells expressing B/bDg, and beads with the highest cell association were isolated and sequenced. Seven novel peptides were identified. Cell uptake analyses and blocking studies revealed that 5 of these peptides displayed specific uptake in B/bDg-overexpressing cells. These candidates displayed nano-/micromolar binding affinity for recombinant B/bDg protein. Further analyses of these candidates using confocal microscopy revealed increased peptide binding/uptake in patient-derived glioma stem cells (GSCs) compared with primary human astrocytes. We plan to incorporate these onto multi-functional BBB-penetrating nanoparticles loaded with imaging agents or a drug payload to translate them into highly selective and efficacious brain cancer theranostic agents.

PS2 – 194

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Bcl-2 Family Member Mcl-1 Expression is Reduced Under Hypoxia by the E3 Ligase FBW7 Contributing to BNIP3 Induced Cell Death In Glioma Cells

Y. Chen[1], E. Henson[1], E. Shome[2], W. Xiao[1], D.D. Eisenstat[2], S.B. Gibson[1]
[1]University of Manitoba, Winnipeg, Manitoba
[2]University of Alberta, Edmonton, AB *eisensta@ualberta.ca*

Mcl-1 is an anti-apoptotic Bcl-2 family member that is often over-expressed in the malignant brain tumour glioblastoma (GBM). It has been previously shown that epidermal growth factor receptors (EGFR) up-regulate Mcl-1 expression contributing to a cell survival response. Hypoxia is a poor prognostic marker in glioblastoma despite the fact that hypoxic regions have areas of necrosis. Hypoxic regions of GBM also highly express the pro-cell death Bcl-2 family member BNIP3, yet when BNIP3 is over-expressed in glioma cells, it induces cell death. The reasons for this discrepancy are unclear. METHODS: Using malignant glioma cell lines +/- hypoxia, gain and/or loss of function assays of BNIP3 or Mcl-1 were performed. BNIP3 and MCL-1 expression was assessed in GBM tumours from adult patients and human gliomas grown as xenografts in immunocompromised mice. RESULTS: Mcl-1 expression is reduced under hypoxia due to degradation by the E3 ligase FBW7 leading to increased hypoxia-induced cell death. This cell death is augmented by EGFR activation leading to increased Mcl-1 expression under hypoxia. Conversely, BNIP3 is over-expressed in hypoxia at times when Mcl-1 expression is decreased. Knocking down BNIP3 expression reduces hypoxia cell death and Mcl-1 expression effectively blocks BNIP3-induced cell death. Of significance, BNIP3 and Mcl-1 are co-localized under hypoxia in glioma cells, GBM tumours and in xenograft glioma tumours expressing mutant EGFR (EGFRvIII). CONCLUSION: These results support that Mcl-1 can block the ability of BNIP3 to induce cell death under hypoxia in GBM tumours