Title

LGG-17. IMPACT OF STROKE AND STROKE RECURRENCE ON LATE MORTALITY AS WELL AS PSYCHOLOGICAL AND SOCIOECONOMIC OUTCOMES IN CHILDHOOD CANCER SURVIVORS

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Pediatric low-grade glioma (PLGG) is one of the most common childhood tumors. If the tumor is located in a brain region that is not accessible for surgical resection, additional therapies are needed. Recent studies highlighted the important role of mTORC and MEK-activation in PLGG. The dual mTORC1/2-inhibitor, TAK-228, and MEK-inhibitor, trametinib, are promising candidates for targeted therapy. We hypothesized that TAK-228 and trametinib would show synergistic effects in vitro and in vivo in PLGG models. We treated the PLGG-derived cell lines Res186 and Res259 and our human MAP-kinase-driven glioma model with TAK-228 and trametinib. Cell growth was investigated using MTT-assay, DNA replication with bromodeoxyuridine (BrdU) assay, and apoptosis through cleaved-caspase-3 (CC-3) staining. Activation of MAPK pathway was detected via Western Blot by pERK and mTOR pathway by pAKT, pS6, and p4E-BP1 to total protein amount, and β-actin. Synergy was calculated with the Chou-Talalay method. Treatment of Res186 and Res259 with TAK-228 or trametinib reduced cell growth and proliferation in a dose- and time-depended manner. The combination of TAK-228 (20nM) and trametinib (100nM) resulted in a synergistic effect in Res259. No synergy was detected for Res186. Staining for CC-3 showed a significant increase for apoptosis in Res259 after treatment with TAK-228 or trametinib (**p<0.01). No positive CC-3 staining was detected in Res186 after drug treatment. MAPK pathway was inactivated in a dose-depend manner after trametinib and mTOR pathway was inactivated after treatment with TAK-228 in both cell lines. Our human neural stem cells similarly showed similar reductions in BrdU incorporation (**p<0.01) and CC-3 (*p<0.05). Our preliminary results show that our used PLGG-models are sensitive to TAK-228 and trametinib treatment. All cell lines showed decreased proliferation at various doses of either inhibitor. Synergy was seen for Res259 cells. We will now investigate both drugs in vivo using a BRAFV600E mutation glioma xenograft.

LGG-14. THE ROLE OF BRAF MUTATIONS IN CLINICAL OUTCOMES IN PEDIATRIC LOW GRADE SPINAL CORD TUMORS

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Over activation of MAPK signaling by oncogenic BRAF occurs in multiple malignancies making it a potential target in oncology. There is also a great body of research evidence to show that BRAF mutations occur in pediatric intracranial tumors. Most low grade gliomas (LGG) of the cerebellum have activation of MAPK/ERK pathway because of KIAA1549-BRAF fusion gene resulting in constitutively active growth signals. Low grade gliomas elsewhere in the brain have lower percentage of gliomas contain a KIAA1549-BRAF fusion. BRAFV600E mutation is seen in a much wider range of both systemic and central nervous tumors including the brain, desmoplastic infantile gangliogliomas, diffuse astrocytomas, gangliogliomas, pleomorphic xanthroastrocytomas, and epitheloid glioblastomas. Alterations in the BRAF gene has been implicated as clinically important in potential outcomes and clinical therapeutic options. We evaluated local clinical and available spinal tumors to determine BRAF mutational status and evaluate if detectable BRAF mutations correlate with clinical outcomes. We evaluated patients with a low grade spinal cord tumor diagnosis made between 1995 and 2016 with a confirmed Grade I or Grade II diagnosis. Twenty-three patients who met all inclusion criteria were evaluated for clinical data including treatment, progression free survival and overall survival. Available tumor samples were evaluated with a cancer gene focused mutation panel to assess for BRAF alterations in addition to other commonly associated LGG alterations in CDKN2A, FGFR, and PIK3CA. Technical difficulties included identification of tumor samples with quality DNA for analysis, particularly associated with early tumor samples which have the greatest clinical follow up. Long-term clinical correlation with mutational changes is particularly important in LGG spinal tumors due to late progression and complications.

LGG-15. CARBOPLATIN IS SYNERGISTIC WITH MAPK INHIBITORS TRAMETINIB AND EVEROLIMUS IN LOW GRADE GLIOMA MODELS

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Pediatric lower grade glioma (LGG) can cause significant morbidity and mortality in patients. Tumors often initially respond to first line chemotherapies, such as carboplatin, but approximately 50% of the time tumors recur and require additional therapy. The discovery that aggressive LGG often have MAPK-ERK pathway, as well as mTOR activation, increases the number of treatment options available, such as trametinib, an ERK inhibitor, or everolimus, an mTORC1 inhibitor. Using preexisting and newly developed models for LGG, our lab sought to determine if there was synergy between carboplatin and the MAPK-ERK and mTOR inhibitors trametinib or everolimus. In the cell lines Res186 (derived from pilocytic astrocytoma grade I), and Res259 (derived from diffuse astrocytoma grade II), we found that the combination of carboplatin with either everolimus or trametinib decreased cellular proliferation in a dose-dependent manner (P<0.05). Both cell lines and drug combinations gave a combination index of <1, indicating synergy. Everolimus sensitized Res259 to carboplatin treatment, despite Res259 being resistant to everolimus treatment alone. Interestingly, the carboplatin and everolimus combination did not increase levels of apoptosis in cells, as measured by cleaved caspase 3 (CC3) staining and probing for cleaved PARP by western blot. We extended our findings to a human neural stem cell glioma model which has MAP kinase activation and confirmed that carboplatin and everolimus combined to suppress proliferation, as measured by BrdU incorporation, greater than either single agent (P<0.001 by ANOVA). Treatments again did not increase apoptosis as measured by CC3 immunofluroescence. Using BT40 patient derived xenografts, a low grade glioma-derived model that harbors a BRAF V600E mutation, preliminary data shows that the combining everolimus with carboplatin had superior growth suppression compared to either drug alone. In conclusion, the combination of carboplatin with everolimus demonstrates combinatorial efficacy in our in vivo and in vitro systems.

LGG-16. LOCATION AND HISTOLOGY DICTATE THE LIKELIHOOD OF MOLECULAR EVENTS IN PEDIATRIC LOW-GRADE GLIOMA

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Over the last decade, a plethora of genetic information in pediatric low-grade gliomas (pLGG) has been uncovered. Clinically, many of these molecular markers have been implicated in aiding in patient diagnosis, predicting prognosis, and stratifying the most effective therapeutic strategy. However, there has yet to be a consensus on what molecular markers to test for, how to test for them, or their incidence across tumour locations and histologies. We compiled a cohort of low-grade gliomas treated at the Hospital for Sick Children. MRI and pathology reviews confirmed tumour location and diagnosis. Molecular testing was conducted using a combination of the QX200 Bio-Rad Droplet-Digital PCR, NanoString nCounter, FISH, and RNA-seq depending on the alteration being tested for and the available material. Our cohort consisted of 480 patients with complete clinical and molecular data. The most frequent alteration in our cohort was KIAA1549-BRAF fusions (37%), followed by BRAF_V600E (20%). BRAF fusion events were most commonly seen in pilocytic and pilomyxoid astrocytoma (67% and 69%, respectively), whereas BRAF_V600E was primarily observed in ganglioglioma and PXA (52% and 85%, respectively). Interestingly, both low grade glioma, NOS and diffuse astrocytoma showed a wide array of molecular events, including BRAF_V600E, BRAF fusions, MYBL1 alterations and H3F3A_K27M. With respect to tumour location, the cerebellum showed significant enrichment for BRAF fusions (80%). In the hemispheres, diencephalon, brainstem and spinal cord, more mutational diversity was seen, with BRAF fusion and BRAF_V600E appearing most frequently, while additional events such as H3F3A_K27M, MYBL1 alterations, and FGFR1 fusions are also present. The work here shows the utility of targeted non-NGS techniques by which hotspot mutations and fusion events can be detected. Further, we show in which histological grades and locations specific events tend to cluster, providing a stepwise procedure by which the most likely molecular marks can be tested for.

LGG-17. IMPACT OF STROKE AND STROKE RECURRENCE ON LATE MORTALITY AS WELL AS PSYCHOLOGICAL AND SOCIOECONOMIC OUTCOMES IN CHILDHOOD CANCER SURVIVORS

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BACKGROUND: Survivors of childhood cancer treated with cranial radiation therapy (CRT) are at high risk for stroke and stroke recurrence. However, the impact of stroke on psychological and socioeconomic outcomes and late mortality is not known. METHODS: Using the Childhood Cancer Survivor Study cohort, mortality rates (IR) per 100 person-years and 95% confidence intervals (CI) were calculated across three time periods: (1) prior to stroke (stroke free); (2) after first stroke but before recurrent stroke (post stroke); and (3) post recurrent stroke. Associations between stroke and psychological (neurocognitive function, emotional distress, health-related quality of life) and socioeconomic outcomes (education, income, employment, marital status, and independent living) were

assessed using multivariable logistic regression adjusted for sex, age at diagnosis, and maximum CRT dose to calculate odds ratios (OR). RESULTS: Among 14,311 5+ year survivors (median age at survey 34.2 years, range 5.6–58.0; median time from diagnosis 26.2 years, range 5.0–38.0) 222 had a stroke ≥ 5years from diagnosis (single n=169; recurrent n=53). Based on 2,021 deaths, the all-cause late mortality rate increased from 0.67 (CI 0.64–0.70) in the stroke-free time period to 3.5 (CI 2.1–6.0) post recurrent stroke. Among 7,205 survivors with longitudinal follow-up, those with history of stroke were: more likely to live with a caregiver (single stroke OR 2.2 (CI 1.4–3.7); recurrent stroke OR 5.1 (CI 1.6–16.0)); limited physical function (single stroke OR 3.0 (CI 1.8–5.1); recurrent stroke OR 10.4 (CI 3.6–30.4)); and increased body pain (single stroke OR 2.0 (CI 1.2–3.4); recurrent stroke OR 3.1, CI 1.1–8.8)). Risk was increased for memory impairment, OR 3.4 (CI 1.1–10.2) and depression OR 4.8 (CI 1.7–13.7) after recurrent stroke. Stroke and stroke recurrence contribute to late mortality and negatively impacts longterm outcome in pediatric cancer survivors. Improved understanding of the pathogenesis of post-CRT stroke is needed to improve stroke prevention strategies.

LGG-18. MOLECULAR ALTERATIONS PREDICT RESPONSE TO CHEMOTHERAPY AND OUTCOME OF PEDIATRIC LOW-GRADE GLIOMA

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BACKGROUND: Pediatric low-grade glioma (pLGG) is the most common childhood brain tumor. Recently, alterations of the RAS/MAPK pathway have been identified as the major driver of PLGG however little is known about prognostic implications. DESIGN/METHODS: We undertook a large population based study of all pLGG diagnosed from 1985–2015. Detection of known pLGG-related fusions was evaluated by NanoString and point mutations BRAF-V600E and H3.3K27M were evaluated using QX200™Droplet Digital™PCR. Results were correlated with outcome and response to chemotherapy. RESULTS: BRAF was found to be altered (KIAA1549-BRAF or BRAF-V600E) in 57% of patients in our pLGG cohort with full clinical and molecular data (n=480). Other alterations accounted for 6% of cases (FGFR1-TACC1(n=9), MYBL1(n=5), H3.3K27M(n=10)). The remaining 37% pLGG cases did not harbor any of evaluated alterations. Additional 134 patients with neurofibromatosis-1(NF1) were included in survival analysis. Kaplan-Meier analysis revealed 10-year PFS 72.3% for NF1, 68.3% for all KIAA1549-BRAF, and 28.4% for BRAF-V600E($p<0.0001$). Among other alterations, H3.3K27M delineated a poor prognostic group with behavior similar to high-grade glioma. All patients with FGFR1-TACC1 and MYBL1 were alive at the time of analysis despite several observed progressions. Furthermore, we evaluated whether differences in survival could be correlated with response to conventional therapy in 92 patients. Change in tumor size at 6 months of chemotherapy differed depending on alteration. 45% of patients with BRAF-KIAA1549 responded to first line chemotherapy, and only 7.5% progressed. Similarly, 35% of NF1 patients responded and none progressed. In contrast, only 15% BRAF-V600E responded and more than half of tumors exhibited growth after six months of chemotherapy. CONCLUSIONS: In contrast to BRAF-V600E, KIAA1549-BRAF defines a group of pLGG patients with excellent prognosis and response to chemotherapy similar to NF1. Biopsy should be mandated in order to predict outcome, response to chemotherapy and evaluate targets for novel therapies.

MEDULLOBLASTOMA/ PRIMITIVE NEUROECTODERMAL TUMORS (PNETS)

MEDU-01. INTERACTION BETWEEN MELK AND EZH2 REGULATES MEDULLOBLASTOMA CANCER STEM-LIKE CELLS PROLIFERATION

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BACKGROUND: Medulloblastoma (MB) is the most common malignant brain tumor in children. Although accumulated research suggests that cancer stem-like cells may play a key role in medulloblastoma tumorigenesis, the molecular mechanisms of proliferation still remain elusive and further investigation can provide a novel application for therapeutic target in MB patients. METHODS: The expression of MELK and EZH2 was detected by tissue microarray analysis with 88 MBs and its association with prognosis was identified. Co-location of MELK and EZH2 in MB CSCs and tissues was studied by using confocal and immunostaining. Immunoblotting analysis following co-immunoprecipitation was performed to check the interaction between MELK and EZH2. Through the loss-of-function study by siRNA, CSCs-drived tumor growth was detected. Then we studied the targeted treatment of MB with MELK and EZH2 inhibitor in vivo to confirm the molecular basis of MELK and EZH2. RESULTS: MELK and EZH2 co-located in the nuclei of MB CSCs and MB with extensive nodularity and large cell/anaplastic differed the staining levels as measured using microarray analysis when compared with the other two subgroups. The proportion of MELK positive staining cells was the potential indicator for the survival. MELK bound and phosphorylated EZH2 and its methylation was induced by EZH2 in MB, which regulated the proliferation of CSCs. MELK and EZH2 depletion by siRNA or treatment of inhibitors attenuated the MB CSCs-derived tumor growth in vivo. CONCLUSION: Interaction between MELK and EZH2 is essential for MB CSCs-drived tumor proliferation, thereby identifying a potential therapeutic strategy for MB patients.

MEDU-02. DIFFERENTIAL EXPRESSION OF FOLATE RECEPTOR 1 IN MEDULLOBLASTOMA AND ITS RELATIONSHIP TO CLINICOPATHOLOGICAL CHARACTERISTICS AND TARGETED THERAPY

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Medulloblastoma is the most common malignant CNS tumor of childhood. High expression of folate receptor 1 (Folr1) was observed in some malignant epithelial tumors. However its expression and the role for clinicopathological significance and targeted therapeutic potential in MB still remain unclear. Currently we have detected the expression of Folr1 in MB and identified its clinical, pathological and radiological values to be considered as a biomarker for diagnosis of MB. Then we studied the targeted treatment of MB with Folr1 targeted cytarabine (Folr1-Ara-C) both *in vitro* and *in vivo*. Folr1 were overexpressed in MB, while the level correlated with pathological subtypes. Folr1 expression was positively correlated with CSF spreading, Ki-67, MMP9, pathological subtypes and serum Folr1. Factors of age, CSF spreading, Ki-67, MMP9, strong Folr1 expression and pathological subtypes were found to be the independent prognostic values for patients with MB. Serum Folr1 showed rational sensitivity and specificity in demonstrating histological types. Folr1-Ara-C led to changes in cellular proliferation and invasion with down-regulation of MMPs proteins and activation of apoptosis *in vitro*. Using mouse xenografts, Folr1-Ara-C suppressed tumor growth and improved survival by MRI and PET/CT. Immunohistochemical analysis showed decreased Ki-67 and MMP9 index suggesting the effects on proliferation and invasion *in vivo*. Folr1 may be considered as a predictive candidate for histological types and serum Folr1 may be a novel non-invasive biomarker. The application of Folr1-Ara-C contributed to be one kind of targeted therapies for MB.

MEDU-03. MEDULLOBLASTOMA GENOMIC SUBGROUP-SPECIFIC OUTCOMES IN IRRADIATED CHILDREN ABOVE 3 YEARS TREATED AT KING FAHAD MEDICAL CITY (KFMC)

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Medulloblastoma (MB) is the most common malignant brain tumor in children. WHO 2016 classify MB into four distinct molecular subgroups. The aim of this study is to characterize the molecular MB subgroups and correlate it with defined clinical outcomes in Middle Eastern children. A retrospective study of newly diagnosed MB in 55 children (>3 year and < 16 year) was collected. All treated at KFMC between 2009–2016. Molecular analysis was done using next generation targeted sequencing