endocrinopathy after treatment for medulloblastoma that can be used for future comparisons.

MEDULLOBLASTOMA (RESEARCH)

MBRS-01. DISSECTING REGULATORS OF THE ABERRANT POST-TRANSCRIPTIONAL LANDSCAPE IN MYC-AMPLIFIED GROUP 3 MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common solid malignant pediatric brain neoplasm, with Group 3 (G3) MB representing the most aggressive subgroup. *MYC* amplification is an independent poor prognostic factor in G3 MB, however, therapeutic targeting of the MYC pathway remains limited and alternative therapies for G3 MB are urgently needed. Here we show that an RNA-binding protein, Musashi-1 (MSI1) is an essential mediator of G3 MB in both *MYC*-overexpressing mouse models and patient-derived xenografts. Unbiased integrative multi-omics analysis of MSI1 function in human G3 MB suggests a paradigm shift beyond traditional gene-based profiling of oncogenes. Here we identify MSI1 as an oncogene in G3 MB driving stem cell self-renewal through stabilization of HIPK1 mRNA, a downstream context-specific therapeutic target for drug discovery.

MBRS-02. BET BROMODOMAIN PROTEIN-KINASE INHIBITOR COMBINATIONS FOR THE TREATMENT OF MEDULLOBLASTOMA

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Recent sequencing studies have implicated many epigenetic regulators in medulloblastoma. The epigenetic reader protein Brd4 has been implicated in various cancers including medulloblastoma. Brd4 controls expression of the medulloblastoma essential genes *MYC* in G3 medulloblastomas, which have poor prognosis as well as *GLI1* and *GLI2* levels in Sonic hedgehog (SHH) driven medulloblastomas, which have intermediate prognosis. Highly selective Brd4 inhibitors have been developed that reduce *MYC*, *GLI1* and *GLI2* levels. These inhibitors have gone into clinical trials for multiple cancer indications including medulloblastoma. However, resistance is common for Brd4 inhibitors warranting combination therapies for improved clinical outcome. We have developed a computational pipeline termed SynergySeq that predicts patient specific combinations of Brd4 inhibitors along with kinase inhibitors. We demonstrate that Brd4-kinase inhibitors robustly reduce proliferation of Shh and MYC driven medulloblastoma cells. Improved efficacy is related to dampening the adaptive kinome reprogramming response that occurs after Brd4 inhibition. Our findings suggest that SynergySeq can be utilized to inform patient selection for clinical trials utilizing Brd4 inhibitors in medulloblastoma and other brain tumors.

MBRS-03. SINGLE NUCLEUS TRANSCRIPTOME PROFILES FROM HUMAN DEVELOPING CEREBELLUM REVEAL POTENTIAL CELLULAR ORIGINS OF MEDULLOBLASTOMA BRAIN TUMORS

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Medulloblastoma (MB) is a highly malignant pediatric brain tumor originating from the cerebellum and brainstem. Identification of molecular subgroups forming this heterogeneous tumor entity was initially achieved from transcriptome characterization and further strengthened using DNA methylation profiling. While subgroup classification improved clinical diagnosis and treatment options, the lack of knowledge of the cell-of-origin for some of the subgroups hinders further treatment improvements. In addition identification of the precise cells of origin for each subgroup could help to understand tumor cell biology. Single cell sequencing is the optimal way to solve this task; recently, there were attempts to uncover putative MB cell-of-origin by using such information obtained from mouse embryonic cerebellum. However, such a comparative strategy can miss important results due to the differences between mouse and human. To solve this issue, we performed global single nucleus sequencing on human cerebellum pre- and postnatal materials across several developmental time points and generated transcriptome profiles from ~200k single cells. We identified known cell types forming the human cerebellum and performed detailed comparison of normal cells to RNA-seq bulk data from MB brain tumors across all subgroups. By selecting an optimal analysis strategy, we verified granule neuron precursors as cells of origin for the SHH MB subgroup. Additionally, we also found other cell types in conjunction with the remaining MB subgroups, suggesting new potential targets for investigation. Notably, this strategy can be further applied to the examination of other brain tumors and has perspectives in medical application.

MBRS-04. MEDULLOBLASTOMA DETECTION BY BLOOD TEST

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INTRODUCTION: Long non coding RNAs (lincRNAs) are functionally defined as transcripts longer than 200 nucleotides in length with no protein coding potential. lincRNA involvement in human cancers etiology is being increasingly proved. Cancer-secreted long non-coding RNAs (lncRNAs) in exosomes are emerging mediators of cancer-host cross talk communication in tumor microenvironments. The ability to monitor and detect tumor markers in real time enables access to tumor biology and may allow highly personalized treatment for each patient. METHODS AND RESULTS: We analyzed RNA sequencing of 64 Medulloblastoma samples and quantified the genome wide long non coding RNAs (lincRNA) expression levels. We identified a lincRNA that is distinctively highly expressed in group 4 (MB4). MB4 expression was further examined in microarray analysis on a larger cohort of medulloblastoma patient samples and a large cohort (n=1405) of patient samples that include normal brain and different brain tumor samples. MB4 proved to be specific and highly expressed in group 4 Medulloblastoma. MB4 was detected in the plasma of medulloblastoma patients with active disease, or subtotal resection. MB4 was not detected in patients that their tumors were resected. MB4 expression is not detected in the serum of medulloblastoma type SHH, penioblastoma, ewing sarcoma and neuroblastoma patients. CONCLUSIONS: We have found that MB4 lncRNA is a highly specific medulloblastoma tumor biomarker and is sensitive and noninvasive biomarker that can be quantified from a blood test. MB4 can be a good diagnostic marker, and in future both may also be a good target for therapy.

MBRS-06. GLI3 INDUCES NEURONAL DIFFERENTIATION IN WNT- AND SHH- ACTIVATED MEDULLOBLASTOMA

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BACKGROUND: We have previously investigated the expression of Gli3, a downstream target of the Sonic Hedgehog pathway, which main function is to suppress Gli1/2 in medulloblastomas. We found that Gli3 is associated with neuronal and glial differentiation in desmoplastic / nodular (D/N) type medulloblastomas (Miyahara *et al.*, Neuropathology, 2013). In the present study, we investigated the expression of Gli3 in molecular subgroups. METHOD: Thirty-one medulloblastomas treated at Niigata University between 1982 and 2013 were studied. Molecular classification into 4 subgroups (WNT-activated, SHH-activated, Group 3 and Group 4) using Nanostring and immunohistochemistry was performed. Furthermore, Gli3 and Gli1 expression in molecular subgroups was assessed using public data bases. RESULTS: Nanostring was considered reliable (confidence > 0.9) in 28 cases. Four cases were classified as WNT-, 5 cases as SHH-activated, 4 cases as Group 3 and 16 cases as Group 4. Gli3 was positive in 7 out of 9 (78%) WNT-/SHH- cases, but positive in only 8 out of 19 (42.1%) non-WNT-/SHH- subgroup cases ($p = 0.1145$, Fisher's exact test). R2 database analysis confirmed that Gli3 was significantly elevated in WNT- and SHH-activated medulloblastoma. Gli1 was elevated in SHH-activated cases but suppressed in WNT-activated cases. IHC analysis revealed that Gli3 was elevated inside nodules showing neuronal differentiation in D/N type

medulloblastoma. Results of single cell RNA analyses were consistent with those of IHC, Nanostring and R2. CONCLUSION: These results suggest that Gli3 is elevated inside the nodules of SHH-activated medulloblastoma, whereas in WNT-activated cases, Gli3 diffusely suppresses HH signaling.

MBRS-08. SONIC HEDGEHOG SIGNALING PRIMES CEREBELLAR GRANULE NEURON PROGENITORS, THE CELL OF ORIGIN FOR MEDULLOBLASTOMA, FOR APOPTOSIS BY INDUCING PRO APOPTOTIC BIM

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Medulloblastomas, unlike other malignant brain tumors, are typically sensitive to radiation therapy, but the mechanisms that mediate this sensitivity are unclear. Cerebellar granule neuron progenitors (CGNPs), the cell of origin for SHH-subgroup medulloblastoma, are also highly sensitive to radiation. In early life, CGNPs proliferate in response to Sonic Hedgehog (SHH) signaling, and hyperactivation of SHH signaling in CGNPs can lead to the development of SHH-subgroup medulloblastoma. We propose that SHH activation induces radiation sensitivity along with tumorigenesis. We have previously shown that the proapoptotic protein BAX is required for radiation sensitivity of both SHH-driven medulloblastomas and CGNPs in mice, and that BCL-xL supplies critical regulation of BAX, preventing spontaneous cell death. Here, we show that SHH signaling increases the radiation sensitivity of CGNPs by inducing the proapoptotic protein BIM. We found that BIM expression depends on SHH activity, and that genetic deletion of *Bim* decreases the radiation-sensitivity of CGNPs. Mechanistically, we show that BIM binds to anti-apoptotic proteins BCL-xL and MCL-1, where it may alter the balance of BAX and BCL-xL interactions. Consistent with our mechanistic model, human medulloblastoma patients with high BIM expression show a better prognosis. Based on these observations, we propose that SHH-induced BIM mediates the typical radiation sensitivity of SHH-driven medulloblastoma. Finding ways to enhance BIM activity may open new opportunities for targeted medulloblastoma therapy.

MBRS-10. QUIESCENT SOX9-POSITIVE CELLS BEHIND MYC DRIVEN MEDULLOBLASTOMA RECURRENCE

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Tumor recurrence is the leading cause of death in medulloblastoma, the most frequent malignant pediatric brain tumor. Recurrence occurs when subpopulations of cancer cells evade standard therapy by acquiring features of immune escape, metastatic spread, and treatment resistance. The transcription factor SOX9 correlated with treatment resistance and dissemination in aggressive Group 3 medulloblastoma. By studying paired primary-recurrent medulloblastoma samples and patient-derived xenograft models, we identified rare SOX9-positive slow-cycling, therapy-resistant tumor cells that accumulate in relapses and in metastases. In an inducible transgenic Group 3 tumor model, doxycycline treatment kills all tumor cells by turning MYC off. However, when MYC expression was redirected to the SOX9 promoter, recurrences from rare, dormant SOX9-positive cells developed with 100% penetrance. Expression profiling revealed that recurrences were more inflammatory, metastatic, and showed elevated MGMT methyltransferase levels which depleted recurrent cells when selectively inhibited. Our model explains how recurrences develop from SOX9-induced quiescence in MYC-driven brain cancer.

MBRS-12. A TRANSPOSON MUTAGENESIS SCREEN IDENTIFIES RREB1 AS A DRIVER FOR GROUP 3 MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common malignant childhood brain tumor. MB can be divided into four major subgroups – WNT, Sonic hedgehog (SHH), Group 3 (G3), and Group 4 (G4) – that exhibit distinct genetic alterations, gene expression profiles, and clinical outcomes. Patients with G3-MB have the worst prognosis, and a deeper understanding of this disease is critical for development of new therapies. Most G3-MBs express high levels of the MYC oncogene, suggesting that MYC plays an important role in tumorigenesis. To identify genes that cooperate with MYC to promote formation of G3-MB, we performed an in vivo mutagenesis screen using mice expressing the Sleeping Beauty (SB) transposon. Cerebellar stem cells from transposon/transposase-expressing mice were infected with viruses encoding Myc, and transplanted into the cerebellum of adult hosts. The resulting tumors were sequenced to identify transposon-targeted genes, and these genes were functionally analyzed to determine whether they could cooperate with Myc to drive G3-MB. These studies identified the transcription factor Ras-responsive element binding protein 1 (Rreb1) as a potent Myc-cooperating gene. Tumors driven by Myc and Rreb1 resemble G3-MB at a histological and molecular level. Moreover, RREB1 is overexpressed in human G3-MB, and knockdown of RREB1 impairs growth of G3-MB cell lines and patient-derived xenografts. Ongoing studies are aimed at identifying the mechanisms by which Rreb1 contributes to tumor growth. Our studies demonstrate an important role for RREB1 in G3-MB, and provide a new model that can be used to identify therapeutic targets and develop more effective therapies for medulloblastoma.

MBRS-13. MIR-1253 POTENTIATES CISPLATIN RESPONSE IN PEDIATRIC MEDULLOBLASTOMA BY REGULATING FERROPTOSIS

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Despite improvements in targeted therapies, few group 3 medulloblastoma patients survive long-term. Haploinsufficiency of 17p13.3 is a hallmark of these high-risk tumors; included within this locus is miR-1253, which has tumor suppressive properties in medulloblastoma. Therapeutic strategies capitalizing on the anti-neoplastic properties of miRNAs can provide promising adjuncts to chemotherapy. In this study, we explored the potentiation of miR-1253 on cisplatin cytotoxicity in group 3 MB. Overexpression of miR-1253 sensitized group 3 MB cell lines to cisplatin, leading to a pronounced downregulation in cell viability and induction of apoptosis. Cisplatin is reported as an inducer of both apoptosis and ferroptosis-mediated cancer cell death. *In silico* analysis revealed an upregulation of several ABC transporters in high-risk MB tumors. When compared to cell lines overexpressing miR-1253, the ABC transporter ABCB7, which regulates both apoptosis and ferroptosis, was revealed as a putative target of miR-1253 with poor survival that may facilitate its chemosensitizing effects by modulating mitochondrial ROS and HIF1α-driven NFκB signaling. We observed high expression of ABCB7 and GPX4, ferroptosis regulators, in MB patients with poor overall survival. MiR-1253 negatively regulated the expression of ABCB7 in Group 3 MB cell lines and induced cytoplasmic ROS and mitochondrial O_2-, suggesting ROS-mediated induction of ferroptosis through regulation of ABCB7 and GPX4. Treatment with ROS and ferroptosis inhibitors rescued miR-1253 transfected cells treated with cisplatin. We conclude that miR-1253 induced ROS and potentiated the ferroptotic effects of cisplatin via targeting miR-1253/ABCB7/GPX4/mtROS axis.

MBRS-14. INTEGRATING CLINICAL AND GENOMIC CHARACTERISTICS IN PEDIATRIC MEDULLOBLASTOMA SUBTYPES IN A SINGLE COHORT IN TAIWAN

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BACKGROUND: Medulloblastoma (MB) was classified to 4 molecular subgroups: WNT, SHH, group 3 (G3), and group 4 (G4) with the demographic and clinical differences. In 2017, The heterogeneity within MB was proposed, and 12 subtypes with distinct molecular and clinical characteristics. PATIENTS AND METHODS: PATIENTS AND METHODS: We retrieved 52 MBs in children to perform RNA-Seq and DNA methylation array. Subtype cluster analysis performed by similarity network fusion (SNF) method. With clinical results and molecular profiles, the characteristics including age, gender, histological variants, tumor location, metastasis status, survival, cytogenetic and genetic aberrations among MB subtypes were identified. RESULTS: In this cohort series, 52 childhood MBs were classified into 11 subtypes by SNF cluster analysis. WNT tumors shown no metastasis and 100% survival rate. All WNT tumors located on midline in 4th ventricle. Monosomy 6 presented in WNT α, but not in β subtype. SHH α and β occurred in children, while SHH γ in infant. Among SHH tumors, α subtype showed the worst outcome. G3 γ showed the highest metastatic rate and worst survival associated with MYC amplification. G4 α has the