MBRS-62. CURAXIN CBL0137 INHIBITS THE VIABILITY OF CANCEROUS CELLS IN PRE-CLINIC MODELS OF MYC-AMPLIFIED MEDULLOBLASTOMA

Jiajia Wang, Baocheng Wang, Yang Zhao, and Jie Ma; XinHua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

Medulloblastoma is a malignant brain tumour that mostly occurs in children, and MYC-amplified medulloblastoma is characterized by pronounced invasiveness and dismal prognosis. There is no effective treatment for Medulloblastoma and its precise pathological mechanism remains obscure. Previous studies indicated that the altered epigenetic machinery manifested in the neoplastic transformation of MYC-amplified MB has become increasingly evident. It is hypothesized that epigenetic genes dependencies associated with small molecule inhibitors that have been approved or are in advanced development may help identify the potential therapeutic targets. By integrating mRNA expression profiles and the corresponding clinicpathologic information of patients suffering from medulloblastoma, and analysing prior CRISPR screening results, we demonstrated that SSRP1 is a negative prognostic factor that functions to stimulate the viability of MB cells. SSRP1 is a subunit of the FACT complex, an important histone chaperone required for transcriptional regulation, DNA replication and damage repair. Its biological effect on tumour proliferation was assessed by using RNA interference and administering CBL0137, a small molecular inhibitor of FACT. Gene expression analysis also demonstrated that CBL0137 selectively downregulated the expression of MYC and NEUROD1. Furthermore, the administration of CBL0137 suppressed tumour growth in mouse xenograft models. This pharmacological method to selectively target MYC transcription was demonstrated in our study, and therefore can be applied as a promising treatment strategy for MYC-amplified medulloblastoma. In Conclusion, we identified an attractive strategy of selectively downregulating MYC transcription by applying inhibitor CBL0137, thereby revealing the potential clinical utility of CBL0137 to improve the prognosis of MYC-amplified medulloblastoma patients.

MBRS-63. THE ROLE OF THE SWI/SNF COMPLEX SUBUNIT SMARCD3 IN MEDULLOBLASTOMA

Brad Poore[1,2], Han Zou[1,2], Vaibhav Sharma[2], Ian Pollack[1,2], and Baoli Hu[1,2]; [1]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [2]UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Medulloblastoma is the most common malignant brain tumor in children, with the Group 3 (G3) having the worst prognosis of the subgroups (WNT, SHH, and Group 4). We aimed to determine the underlying differences between G3 and the other subgroups, with an emphasis on genes that control the epigenome for developing effective treatments for patients with this disease. To this end, we found that G3 has elevated expression of the SWI/SNF subcomponent, SMARCD3 (P<0.001), which serves to guide the SWI/SNF complex to different genomic regions through interactions with various transcription factors. However, little is known about function of SMARCD3 in cancer, particularly in medulloblastoma. Clinically, elevated SMARCD3 mRNA resulted in a poorer prognosis in medulloblastoma patients (P<0.0001), which was further validated in 63 patient tumors by immunohistochemical staining for SMARCD3. Interestingly, tumors that had metastasized often had elevated expression of SMARCD3, in all subgroups of medulloblastomas (P<0.0001) and G3 only (P<0.01) based on analyzing multiple published databases. An orthotopic mouse model further supported that SMARCD3 is highly expressed in metastatic tumors compared to primary tumors. Importantly, CRISPR-CAS9-mediated SMARCD3 deletion decreased cell migration in medulloblastoma cell lines. Mechanistically, SMARCD3 deletion led to decreased H3K27me3, suggesting that SMARCD3 may cooperate with PRC2 in regulation of gene expression. Together, our results indicate that SMARCD3 plays a significant role in the development of metastatic dissemination in medulloblastoma, especially in the G3 subgroup. Thus, targeting the SMARCD3-containing SWI/SNF Complex may effectively prevent tumor dissemination and improve clinical outcomes in children with medulloblastoma.

MBRS-64. STUDY OF ARGININE METHYL TRANSFERASES IN MEDULLOBLASTOMA

Donghang Cheng[1], Pete Taylor[1], Monica Gireud[1], Shinji Maegawa[1], Yanwen Yang[1], Sadhan Majumder[2], and Vidya Gopalakrishnan[1,3]; [1]Department of Pediatrics, University of Texas, MD Anderson Cancer Center, Houston, TX, USA, [2]Department of Genetics, University of Texas, MD Anderson Cancer Center, Houston, TX, USA, [3]Department of Molecular and Cellular Oncology, University of Texas, MD Anderson Cancer Center, Houston, TX, USA

Medulloblastoma is the most common malignant pediatric cancer and a leading cause of childhood cancer-related mortality. There is dire need for new therapies. Molecular sub-classification of medulloblastomas has identified chromatin modifiers as potential drivers of tumorigenesis. Expression of the *RE1* Silencing Transcription Factor (REST), a hub for assembly of repressive chromatin remodelers and a known regulator of neurogenesis, is elevated in a subset of human sonic hedgehog (SHH) subgroup medulloblastomas, and is associated with poor prognosis. Using a novel transgenic mouse model, we showed REST to be a driver of medulloblastoma development. Surprisingly, our studies also revealed a role for REST in promoting proliferation of granule cell progenitors (GCPs), the cells of origin of SHH-driven medulloblastoma, and a concomitant loss of telomeres and increased genomic instability. We performed a gain of function screen using a library of chromatin modifiers to understand the mechanism by which proliferative potential was maintained, despite the loss of telomeres. This screen identified the Protein Arginine Methyltransferase 6 (*PRMT6*) as a high confidence hit. PRMT6 upregulation caused a reduction in CDKN2A, an important regulator of replicative senescence. Evasion of senescence is frequently implicated in tumor progression. Using a chemical screen, we also identified a novel, selective, reversible and competitive inhibitor of PRMT6. The consequence of genetic and pharmacological inhibition of PRMT6 on cell proliferation and senescence will be reported. Thus, our studies are the first to demonstrate a role for arginine methyl transferase family of chromatin modifiers in medulloblastoma genesis.

MBRS-65. FBXW7 ACTS A TUMOR SUPPRESSOR IN MYC-DRIVEN MEDULLOBLASTOMA BY CONTROLLING A FEED-FORWARD REGULATORY LOOP OF PLK1 AND MYC

Dong Wang[1], Angela Pierce[1], Bethany Veo[1], Susan Fosmire[1], Krishna Madhavan[1], Ilango Balakrishnan[1], Sujatha Venkataraman[1,2], and Rajeev Vibhakar[1,2]; [1]University of Colorado School of Medicine, Aurora, CO, USA, [2]Morgan Adams Pediatric Brain Tumor Research Program, Aurora, CO, USA

Group 3 medulloblastoma (MB) is often accompanied by MYC amplification and has a higher rate of metastatic disease. So, it is critical to have more effective therapies for high MYC expressing sub-groups. Here we report that FBXW7, a substrate recognition component of the SKP1-CUL1-Fbox (SCF) E3 ligase, interacts with and targets c-MYC for polyubiquitination and proteasomal degradation. FBXW7 shows lower expression level in MYC-driven MB compared with other MB subgroups suggesting activity as a tumor suppressor. Genomic deletion or mutation of *Fbxw7* has frequently been identified in many human cancers but not in MB. We demonstrate that overexpression of *Fbxw7* in MB cells induces apoptosis and suppresses proliferation *in vitro* and *in vivo*. Both phospho-deficient (T205A) and phosphomimetic aspartic acid (T205D) mutants deactivate its tumor suppressor function suggesting a conformational change of its protein structure. Mechanistically, PLK1 kinase specifically phosphorylates FBXW7 and promotes its auto-polyubiquitination and proteasomal degradation, counteracting FBXW7-mediated degradation of oncogene substrates, including c-MYC and PLK1. Chip-Seq results show stabilized c-MYC in turn directly activates PLK1 and FBXW7 transcription, constituting a feedforward regulatory loop. Co-immunoprecipitation demonstrates that FBXW7 directly binds to PLK1 and c-MYC, facilitating their protein degradation by promoting the ubiquitination of both proteins. Furthermore, we show that FBXW7 protein can be stabilized by various kinase inhibitors, proposing a mechanism of kinase-targeted agents to treat MYC-driven MB. These results collectively demonstrate how kinase inhibition stabilizes the tumor suppressor FBXW7 in MYC-driven MB, thus revealing an important function of FBXW7 in suppressing MB progression.

MBRS-66. COST-EFFECTIVE METHOD TO INCORPORATE MOLECULAR CLASSIFICATION OF MEDULLOBLASTOMA INTO A LATIN-AMERICAN CLINICAL TRIAL

Sidnei Epelman[1,2], Luis Henrique Sakamoto[1,2], Juliana Monte Real[2], Vijay Ramaswamy[3], Gregorio Pereira[2], Simone dos Santos Aguiar[4], and Flavia Caldeira Queiroz[2]; [1]Santa Marcelina Hospital / Department of Pediatric Oncology, Sao Paulo, SP, Brazil, [2]Nucleo Avancado de Pesquisa em Oncologia / Santa Marcelina Faculty, Sao Paulo, SP, Brazil, [3]The Hospital for Sick Children, Toronto, ON, Canada, [4]Santa Marcelina Hospital, Sao Paulo, SP, Brazil

BACKGROUND: It is now widely accepted that medulloblastoma actually comprises four distinct molecular subgroups, requiring specific treatment strategies. Implementing routine subgrouping in a time and cost effective manner is a major challenge in Latin America, particularly the development of molecular informed clinical trials. Herein we describe the feasibility of reliable and rapid molecular stratification using a qPCR method integrated with immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH – c-myc, monosomy 6) from heterogeneously fixed, low-quality FFPE samples across Latin America. RESULTS: Fifty-four FFPE samples were classified according to histologic and molecular criteria. Classic medulloblastoma was found in 53.7%, desmoplastic/extensive nodularity in 24.1%, NOS in 16,7% and anaplastic in 5,6%. IHC