BIOL-10. DISTRIBUTION AND VULNERABILITY OF TRANSCRIPTIONAL OUTPUTS ACROSS THE GENOME IN MYC-AMPLIFIED MEDULLOBLASTOMA CELLS

Rui Yang[1], Wenzhe Wang[1], Meichen Dong[2], Kristen Roso[1], Xuhui Bao[1], Christopher Pirozzi[1], Darell Bigner[1], Hai Yan[1], David Ashley[1], Vasyl Zhabotynsky[2], Fei Zou[2], and Yiping He[1]; [1]Duke University, Durham, NC, USA, [2]UNC-CH, Chapel Hill, NC, USA

Myc plays a central role in tumorigenesis by orchestrating the expression of genes essential to numerous cellular processes. While it is well established that Myc functions by binding to its target genes to regulate their transcription, the distribution of the transcriptional output across human genome in Myc-amplified cancer cells, and the susceptibility of such transcriptional outputs to therapeutic interferences remain to be fully elucidated. Here, we analyze the distribution of transcriptional outputs in Myc-amplified medulloblastoma (MB) cells by profiling nascent total RNAs within a temporal context. This profiling reveals a major portion of transcriptional action in these cells was directed at the genes fundamental to cellular infrastructures, including rRNAs and particularly those in the mitochondrial genome (mtDNA). Notably, even when Myc protein was depleted by as much as 80%, the impact on transcriptional outputs across the genome was limited, with notable reduction mostly in genes of involved in ribosomal biosynthesis, genes residing in mtDNA or encoding mitochondria-localized proteins, and those encoding histones. In contrast to the limited direct impact of Myc depletion, we found that the global transcriptional outputs were highly dependent on the activity of Inosine Monophosphate Dehydrogenases (IMPDHs), rate limiting enzymes for de novo guanine nucleotide synthesis and whose expression in tumor cells was positively correlated with Myc's expression. Blockage of IMPDHs attenuated the global transcriptional outputs with a particularly strong inhibitory effect on the aforementioned infrastructure genes, which was accompanied by the abrogation of MB cell's proliferation in vitro and in vivo. Together, our findings reveal a real time action of Myc as a transcriptional factor in tumor cells, gain new insight into the pathogenic mechanism underlying Myc-driven tumorigenesis, and support IMPDHs as a therapeutic vulnerability in MB cells empowered by a high level of Myc oncoprotein.

BIOL-11. THE ROLE OF ABERRANT EXPRESSION OF PRDM6 IN THE DEVELOPING CEREBELLUM AND IN GROUP 4 MEDULLOBLASTOMA

Christin Schmidt, Annika Carlson, William Weiss, and Bjoern Schwer; University of California, San Francisco, San Francisco, CA, USA

Group 4 medulloblastoma is the most common medulloblastoma subgroup with an intermediate prognosis and a high incidence of metastasis and late-onset relapse cases. Despite several comprehensive genomic studies in medulloblastoma, Group 4 medulloblastomas lack a unifying oncogenic driver and treatment targets. This subgroup is characterized by recurrent genetic alterations in chromatin modifiers, amplification of stemness genes, and enhancer hijacking events. 17% of Group 4 medulloblastoma cases are characterized by enhancer hijacking through tandem duplication of *SNCAIP*, resulting in high expression of PRDM6, a putative transcriptional repressor and histone methyltransferase. PRDM6 amplified medulloblastoma cases show additional mutations in other chromatin regulators, such as KDM6A, KMT2C and KMT2D, ZMYM3, and high MYCN expression. In this project, we investigate the impact and oncogenic potential of sustained PRDM6 expression in early neural stem cell populations and the developing mouse cerebellum. We drive expression of PRDM6 in human iPSC-derived neuroepithelial stem cells (NESCs) with and without high MYCN expression to study its implications in tumorigenesis. To test for tumor growth *in vivo* and changes in tumor progression as a function of PRDM6 activity, NESCs are injected into the cerebellum of adult mice. In order to elucidate impact of PRDM6 activity during embryonic cerebellar development, we also introduce PRDM6 expression into mouse embryonic stem cells (ESCs) for analysis via a new, *in vivo* cerebellar blastocyst complementation model. The latter approach is designed to ablate and repopulate early granule neural precursor cells in the embryonal cerebellum with progenitors derived from injected PRDM6-ESCs and thus to recapitulate pre- and postnatal cerebellar development *in vivo*. Together, our studies aim to understand the role of PRDM6 during normal cerebellar development and tumorigenesis and advance the understanding of the genetic drivers for Group 4 medulloblastoma.

EMBRYONAL TUMORS

EMBR-01. CLASS I HDAC INHIBITORS AND PLK1 INHIBITORS SYNERGIZE IN *MYC*-AMPLIFIED MEDULLOBLASTOMA

Gintvile Valinciute[1,2], Jonas Ecker[1,2], Florian Selt[1,2], Thomas Hielscher[3], Christin Schmidt[4], Romain Sigaud[1,2], Johannes Ridinger[1,2], Charlotte Gatzweiler[1,2], Daniel Picard[5,6], Sina Oppermann[1,2], Mirjam Blattner-Johnson[1,7], David T. W. Jones[1,7], Ina Oehme[1,2], Marcel Kool[1,8], Marc Remke[5,6], Stefan M. Pfister[1,4], Olaf Witt[1,2], and Till Milde[1,2]; [1]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany, [2]Clinical Cooperation Unit Pediatric Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, [3]Division of Biostatistics, German Cancer Research Center (DKFZ), Heidelberg, Germany, [4]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany, [5]Department of Pediatric Oncology, Hematology and Clinical Immunology, Duesseldorf University Hospital, Duesseldorf, Germany, [6]German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany, [7]Pediatric Glioma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany, [8]Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

Background: Medulloblastoma (MB) is one of the most common malignant pediatric CNS tumors. Patients with Group 3 MBs harboring *MYC* amplification exhibit low survival rates. Surviving patients suffer from therapy-induced sequelae, which calls for new targeted therapy strategies. We and others have previously shown the sensitivity of *MYC*-amplified MB to class I histone deacetylase (HDAC) inhibition. After demonstrating that the MYC target gene *PLK1* is significantly downregulated upon class I HDACi treatment, we hypothesized that inhibition of both HDACs and PLK1 could have synergistic effects. Methods: Cell metabolic activity changes upon HDAC and PLK1 inhibitor treatment were measured in *MYC*-amplified and non-amplified MB cell lines, as well as in an additional *MYC*-inducible cell line. The interaction effect of both inhibitors was determined by computation of the combination index (CI) using the Chou-Talalay method. Results were validated assessing cell viability, cell cycle, and apoptosis induction. Transcription profile changes after combination treatment were evaluated. Results: *MYC*-amplified MB cell lines were more sensitive than non-amplified cell lines to PLK1i treatment, showing IC50 in clinically achievable concentration ranges. Inhibition of class I HDACs and PLK1 synergistically reduced cell metabolic activity in lower concentrations in *MYC*-amplified compared to non-amplified MB cell lines. We also observed a significant loss of viability and cells in G1 phase, as well as induction of apoptosis after combination treatment in *MYC*-amplified cells. MYC target gene sets were significantly downregulated in the *MYC*-amplified cell line HD-MB03 after treatment with combination. We demonstrated reduction of MYC protein levels upon PLK1i treatment. *In vivo* evaluation of combination treatment using orthotopic Group 3 *MYC*-amplified MB PDX models is ongoing. Conclusion: Our data suggest that *MYC*-amplification is a predictive marker for PLK1i treatment in MB. The combination of HDACi and PLKi could be a candidate therapy for future clinical trials for *MYC*-amplified group 3 MB.

EMBR-02. OLIG2 REPRESENTS A PROGNOSTIC MARKER AND THERAPEUTIC TARGET IN MYC-AMPLIFIED MEDULLOBLASTOMA RELAPSE AND METASTASIS

Zhenhua Xu[1], Najiba Murad[1], Samuel Rivero-Hinojosa[1], Ulrich Schüller[2], Peng Zhang[3], Xiao Liu[1], Brian Rood[1], Roger Packer[1], and Yanxin Pei[1]; [1]Children's National Medical Center, Washington, DC, USA, [2]University Medical CenterHamburg-Eppendorf, Hamburg, Germany, [3]University of Maryland, Baltimore, MD, USA

Medulloblastoma (MB) is one of the most common malignant pediatric brain tumors. Among the multiple MB subtypes, MB with *MYC* amplification confers an extremely poor prognosis with an overall survival rate of less than 30%. Relapse is often mediated by a small population of therapy-resistant tumor cells which expand and ultimately progress to lethal tumors. Moreover, *MYC*-amplified MB exhibits a high incidence of leptomeningeal metastases. Approximately one-third of patients with *MYC*-amplified MB present with metastases and nearly all have this complication at relapse. Metastatic *MYC*-amplified MB is highly fatal. As such, our ability to effectively treat *MYC*-amplified MB is largely dependent on our capacity to eradicate the therapy resistant tumor cells, particularly the metastatic tumor cells. The development of clinically effective therapies for this disease will be facilitated by the identification of therapy-resistant tumor cell populations and their molecular signatures involved in tumor metastasis and relapse. Using patient-derived xenograft (PDX) mouse models, we recently discovered that a subset of *MYC*-amplified MB tumors with strong OLIG2 expression ($OLIG2^{-high}$) is resistant to radiation and prone to metastasize, whereas *MYC*-amplified MB tumors without OLIG2 expression ($OLIG2^{-low}$) are sensitive to radiation without dissemination. Irradiation of $OLIG2^{-high}$ tumors led to either a small number of quiescent $OLIG2^{-}$ cancer stem-like cells (CSLCs) remaining in the cerebellar bed or to the dissemination of highly proliferative $OLIG2^{+}$ tumor cells along the leptomeninges. All mice harboring these radioresistant CSLCs succumbed to relapse. Further studies demonstrated that the quiescent $OLIG2^{-}$ CSLCs did not contribute to tumor recurrence directly, while elimination of $OLIG2^{+}$ radioresistant CSLCs with a small molecule OLIG2 antagonist significantly prevented metastatic recurrence, delayed tumor growth and prolonged animal survival. Thus, our

studies provide new insights into the role of OLIG2 in radiotherapy resistance and metastasis in *MYC*-amplified MB and propose a novel therapeutic approach to treating metastatic *MYC*-amplified MB.

EMBR-03. PINEOBLASTOMA: A POOLED OUTCOME STUDY OF NORTH AMERICAN AND AUSTRALIAN THERAPEUTIC DATA

Jordan Hansford[1,2], Jie Huang[3], Andrew Dodgshun[4], Bryan Li[5,6], Eugene Hwang[7], Sarah Leary[8], Amar Gajjar[9], Katja Von Hoff[10], Raelene Endersby[11,12], Olivia Wells[13], Alison Wray[1], Rishi Kotecha[14,15], David Raleigh[16,17], Schuyler Stoller[18], Sabine Mueller[18], Steven Schild[19], Pratiti Bandopadhayay[20], Maryam Fouladi[21], Eric Bouffet[5], Annie Huang[5,6], Arzu Onar[22], and Nicholas Gottardo[14,15]; [1]Royal Children's Hospital; Murdoch Children's Research Institute, Melbourne, VIC, Australia, [2]University Melbourne, Melbourne, VIC, Australia, [3]Children's Oncology Group Department of Biostatistics, Memphis, TN, USA, [4]Christchurch Children's Haematology/Oncology Centre, Christchurch, New Zealand, [5]Division of Hematology/Oncology, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada, [6]Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada, [7]Children's National, Division of Oncology, Washington DC, USA, [8]Fred Hutchinson Cancer Centre, Seattle, WA, USA, [9] St. Jude Children's Research Institute, Memphis, TN, USA, [10]Department of Pediatric Oncology and Haematology, Charite University Medicine, Berlin, Germany, [11]Brain Tumour Research Program, Telethon Kids Institute, Nedlands, WA, Australia, [12]University of Western Australia, Perth, WA, Australia, [13]Royal Children's Hospital, Melbourne, VIC, Australia, [14]Telethon Kids Cancer Centre, Telethon Kids Institute, University of Western Australia, Perth, WA, Australia, [15]Department of Clinical Haematology, Oncology and Bone Marrow Transplantation, Perth Children's Hospital, Perth, WA, Australia, [16]Department of Radiation Oncology, UCSF, San Francisco, CA, USA, [17]Department of Neurologic Surgery, UCSF, San Francisco, CA, USA, [18]Department of Pediatric Oncology, UCSF, San Francisco, CA, USA, [19]Mayo Clinic, Department of Radiation Oncology, Phoenix, AZ, USA, [20]Boston Children's Hospital, Dana Farber Children's Institute, Boston, MA, USA, [21]Nationwide Children's Hospital, Department of Neuro-Oncology, Columbus, OH, USA, [22]Children's Oncology Group Department of Biostatistics, Memphis, TN, Canada

Background: Pineoblastoma (PB) is a rare embryonal brain tumour most often diagnosed in young children. To date, no clinical trials have been conducted specific to pediatric PB. Collaborative studies performed over the past 30 years have included PB in studies accruing for other embryonal tumours, primarily medulloblastoma (MB), but also including the entity formerly known as CNS-PNET and atypical teratoid rhabdoid tumors. Each of these studies have included only a small number of children with PB, making clinical features difficult to interpret and determinants of outcome difficult to ascertain. Patients and Methods: Published centrally reviewed series with sufficient treatment and outcome data from North American and Australian cases were pooled. To investigate associations between variables, Fisher's exact and Wilcoxon-Mann-Whitney tests, and Spearman correlations were used as appropriate. Kaplan-Meier plots, log-rank tests, and Cox proportional hazards models were used in survival analysis. Results: We describe a 30-year review of the reported clinical features of PB and a pooled centrally reviewed, cohort analysis of cases (n=178) from the Children's Oncology Group (COG) (n=82) groups and several published, centrally reviewed institutional series (n=96). We find young children <3 years of age have a dramatically poorer outlook compared to older children (5-year OS 16.2% +/- 5.3% vs 67.3% +/- 5%) confirming new and novel approaches are needed in future clinical trials for this at risk group. Interestingly, male gender was predictive of worse outcome possibly suggestive of gender specific subgroup risks that needs validation in future studies. Assessment of radiation therapy is not possible as the vast majority of children under age three did not receive any form of radiation therapy. Conclusion: Given the relative scarcity of this tumor and the emerging data on subgroups of pineoblastoma, prospective, collaborative international studies will be vital to improving the long-term survival of these patients.

EMBR-04. BET INHIBITION TARGETS RADIOTHERAPY RESISTANCE IN H3K27ME3-DEFICIENT GROUP 3 MEDULLOBLASTOMA

Nishanth Gabriel[1], Kumaresh Balaji[1,2], Matthew Inkman[1], Kay Jayachandran[1], Jin Zhang[1], Sonika Dahiya[1], and Michael Goldstein[1]; [1]Washington University School of Medicine, St. Louis, MO, USA, [2]Rush University School of Medicine, Chicago, IL, USA

Medulloblastoma has been categorized into four subgroups based on genetic, epigenetic and transcriptional profiling. However, molecular pathways determining radiotherapy response in this tumor remain elusive. Here, we investigated the role of the EZH2-dependent histone H3K27 tri-methylation in radiotherapy response in medulloblastoma. We demonstrate that 47.2% of group 3 and 4 medulloblastoma patients have H3K27me3-deficient tumors. Loss of H3K27me3 was associated with a radioresistant phenotype, high relapse rates and poor overall survival. We show that an epigenetic switch from H3K27me3 to H3K27ac occurs at specific genomic loci in H3K27me3-deficient medulloblastoma cells altering the transcriptional profile. The resulting up-regulation of EPHA2 (ephrin type-A receptor 2) stimulates an excessive activation of the pro-survival AKT signaling pathway leading to radiotherapy resistance. We show that BET inhibition targets radiation resistance in H3K27me3-deficient medulloblastoma by suppressing H3K27ac levels, blunting EPHA2 overexpression and mitigating the excessive AKT signaling. Additionally, BET inhibition sensitizes medulloblastoma cells to radiation by enhancing apoptotic response through suppression of Bcl-XL and up-regulation of Bim expression. Our work demonstrates a novel mechanism of radiation resistance in medulloblastoma and identifies an epigenetic marker predictive of radiotherapy response. Based on these findings we propose an epigenetically guided treatment approach targeting radiotherapy resistance in medulloblastoma patients.

EMBR-05. THE TENTATIVE APPLICATION OF EN BLOC CONCEPT IN THE PEDIATRIC BRAIN TUMOR: EXPERIENCE FROM A LARGE PEDIATRIC CENTER IN CHINA

Liangliang Cao and Jie Ma; Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, Shanghai, China

Background: The less allowable blood loss and tolerance of intraoperative blood loss of children lead to the high rate of massive blood transfusion in the treatment of brain tumor. The surgical concepts of en bloc resection may contribute to the improvement of brain tumor resection. Objective: To investigate the effects of en bloc concept on short outcomes of pediatric brain tumors and factors associated with the application of en bloc concept. Methods: According to the surgical concept involved, the patients were divided into three subgroups-complete en bloc concept, partial en bloc concept and piecemeal concept. The matching-comparison (piecemeal group and en bloc group formed from the first two subgroups) was conducted based on age, tumor location, lesion volume, and pathological diagnosis to investigate effect of the en bloc concept on the short-term outcomes. Then the patient data after January 2018, when the en bloc concept was routinely integrated into brain tumor surgery in our medical center, were reviewed and analyzed to find out the predictors associated with the application of en bloc concept. Results: In the en bloc group, the perioperative outcomes, including hospital stay (p=0.001), PICU stay (p=0.003), total blood loss(p=0.015), transfusion rate(p=0.005) and complication rate(p=0.039), were all significantly improved. The multinomial logistic regression analysis showed that tumor volume and imaging features, like bottom vessel, encasing nerve or pass-by vessel, finger-like attachment, ratio of "limited line" and ratio of "clear line" remained independent factors for the application of en bloc concept in our medical center. Conclusion: This study supports the application of complete or partial en bloc concept in the pediatric brain tumor surgery referring to the preoperative imaging features, and compared with piecemeal concept, en bloc concept can improve the short outcomes without significant increases in neurological complication. Large series and Additional supportive evidence are still warranted.

EMBR-06. EFFECTIVE INHIBITION OF MYC-AMPLIFIFIED GROUP 3 MEDULLOBLASTOMA BY FACT-TARGETED CURAXIN DRUG CBL0137

Jiajia Wang and Jie Ma; Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, Shanghai, China

Medulloblastoma (MB) is the most common malignant pediatric brain tumor that can be categorized into four major molecular subgroups. Group 3 MB with MYC amplifification (MYCamp-G3-MB) has been shown to be highly aggressive and exhibited worst prognosis, indicating the need for novel effective therapy most urgently. A few epigenetic targeted therapeutic strategies have recently been proven to effectively treat preclinical models of MYCamp-G3-MB, including BET inhibition, HDAC inhibition and SETD8 inhibition, unveiling a promising direction for further investigation. In this study, we carried out systemic bioinformatic analyses of public-available MB datasets as well as functional genomic screening datasets of primary MYCamp-G3-MB lines to search for other potential therapeutic targets within epigenetic modulators. We identififfied SSRP1, a subunit of histone-chaperone FACT complex, to be the top drug target candidate as it is highly cancer-dependent in whole-genome CRISPR-Cas9 screening across multiple MYCamp-G3-MB lines; significantly upregulated in MYCamp-G3-MB compared to normal cerebellum and most of the rest MB subtypes; its higher expression is correlated with worse prognosis; and it has a blood-brain-barrier penetrable targeted drug that has entered early phase human clinical trials already. Then we utilized RNA-interference approach to verify the cancer-dependency of SSRP1 in multiple MYCamp-G3-MB lines and further confiffirmed the therapeutic effifficacy of FACT-targeted curaxin drug CBL0137 on treating preclinical models of MYCamp-G3-MB in vitro and in vivo, including an orthotopic intracranial xenograft model. Mechanistically, transcriptome analyses showed