MBRS-19. BIOCHEMICAL AND ONCOGENIC CHARACTERIZATION OF HOTSPOT KBTBD4 MUTATIONS IN MEDULLOBLASTOMA

Hilmarie Muniz-Talavera, Jennifer Hadley, Paul A Northcott
work investigated several potential innovative peptidomimetics that specifically targeted NRP-1 and showed that MR438 has a good affinity for NRP-1. This small molecule decreased the self-renewal capacity of MB stem cells for the first time as assessed by increased the invasive abilities of DAOY and D283 cells, while NRP-1 expression and cancer stem cell markers decreased at the same time. Possible molecular mechanisms were explored and showed that phosphorylation of AKT and ERK proteins in PKM2/ACT and MAPK pathways significantly decreased for DAOY-MS cells after treatment. Finally, our results highlighted that targeting NRP-1 with MR438 is a potential new strategy to differentiate MB stem cells and improve chance to cure.

MBRS-18. ALK EXPRESSION AT THE PROTEIN LEVEL IS A MARKER FOR THE DIFFERENTIATION DIAGNOSIS OF THE WNT-ACTIVATED TYPE OF PEDIATRIC MEDULLOBLASTOMA

Marija Lastowska1, Joanna Trubicka1, Magdalena Niemirza2, Magdalena Pasionczak-Abubakr3, Agnieszka Karkucinska-Wieczkowska1, Magdalena Kaleta1, Monika Drogoszewicz1, Magdalena Tarasinska1, Marta Perek-Polnik1, Adam Kretowski1,2, Bozenna Dembowska-Baginska1, Wieslawa Grajowska1, Maciej Pronicki1, and Ewa Matysi1. Department of Pathology, The Children’s Memorial Health Institute, Warsaw, Poland, 2Clinical Research Centre, Medical University of Białystok, Białystok, Poland, 3Clinic of Oncology, The Children’s Memorial Health Institute, Warsaw, Poland, 4Department of Endocrinology, Diabetology and International Pediatrics, Medical University of Białystok, Białystok, Poland, 5Department of Experimental and Clinical Pathology, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

OBJECTIVES: Previous analyses indicated that expression of ALK at the RNA level is correlated with the WNT-activated type of medulloblastoma. Therefore, we investigated if ALK expression at the protein level may serve as a useful marker for identification of this type of medulloblastoma. METHODS: Tumors from 134 patients were studied at diagnosis by immunohistochemistry using antibody ALK clone D53F #3633 (Cell Signaling). Consensus scoring was applied as the following: 0 (negative), 1 (equivocal <10%), 2 (low expression 10%-50%), 3 (moderate expression 50%-100%), 4 (high expression >100%) of positive tumor cells. Transcriptional subtypes of medulloblastoma tumors were established in all cases. Thirty infratentorial ependymal tumors were also investigated to determine if ALK protein expression may serve as a marker for differential diagnosis. RESULTS: All 19 WNT tumors were positive for ALK expression and in all but one positive reaction was present in >50% of tumor cells (score 4+). Only four ALK positive tumors were detected among the remaining 85 tumors: one tumor with a score 2+ in Group 4 and three tumors with a score 1+ (one in Group 3 and two in SHH group). Therefore, expression of ALK is strongly associated with the WNT type of disease (p=0.0001). By contrast, none of the analyzed ependymal tumors displayed positive ALK reaction. CONCLUSION: The results indicate that ALK expression at the protein level may serve as a marker for differential diagnosis for the WNT-activated type of medulloblastoma. Funded by National Science Centre, Poland (2016/21/B/NZ2/01785 and 2016/23/B/NZ2/03064).

MBRS-19. BIOCHEMICAL AND ONCOGENIC CHARACTERIZATION OF HOTSPOT KBTBD4 MUTATIONS IN MEDULLOBLASTOMA

Hilmar Munte-Talavera, Jennifer Hasley, and Paul A. Northcott. St. Jude Children’s Research Hospital, Memphis, TN, USA

Medulloblastoma (MB) is the most common malignant childhood brain tumor. Our recent analysis of the MB genomic landscape in a cohort of 500 patients disclosed 182 recurrent somatic hotspot mutations targeting KBTBD4, a poorly characterized member of the BTB-Back-Kelch domain family of proteins. Based on homology with other family members, KBTBD4 is predicted to function as a substrate adaptor for E3 ubiquitin ligases, facilitating substrate recruitment via its C-terminal Kelch domain. KBTBD4 mutations occurred exclusively in Group 3 and Group 4 patients as in-frame insertions clustered in the Kelch domain, suggesting these variants may disrupt physiological KBTBD4-substrate interactions. Herein, we used a combination of advanced proteomic and gene targeting to determine the normal physiological function of KBTBD4 and elucidate its role in MB pathogenesis. IP-MS/MS results identified proteins associated with the ubiquitin-proteasome system (UPS), including E3 ubiquitin ligases (CUL3, CHIP, UBR4), ubiquitin receptor-associated proteins (CAGU1, RP27A), as well as chaperone-associated proteins (HS9P0, TCP1, BAG2, NUD3C3). Candidate interacting partners were subsequently confirmed through co-IP/Western blot experiments, substantiating preliminary hypotheses related to KBTBD4 function. To investigate the role of mutant KBTBD4 in MB progression, we have targeted KBTBD4 in MB PDX models harboring prototypic insertions. Parallel studies encompassing a complete phenotypic characterization of KBTBD4 knock-out and knock-in mice carrying MB-specific mutations will further validate KBTBD4 as a bona fide MB driver gene, and mechanistically dissect how KBTBD4 Kelch domain mutations promote tumorigenesis. These studies will provide new insights into MB biology with the potential to disclose novel therapeutic vulnerabilities for treating mutant KBTBD4-driven MB.

MBRS-20. KETOLYTIC AND GLYCOLYTIC ENZYMATIC EXPRESSION IN PAEDIATRIC MEDULLOBLASTOMAS: IMPLICATION FOR KETOGENIC DIET THERAPY

Lisa C D Storey, Zacharias de Beer, George Lockwood, Simon Paine, and Richard G Grundy. University of Nottingham, Nottingham, Nottinghamshire, UK

Medulloblastomas are the most common malignant brain tumour of childhood. Long term survival is approximately 75%, however, therapy often results in disabling outcomes and reduced quality of survival. One potential strategy to improve outcome, whilst reducing side effects, is exploiting the metabolic differences between normal and tumour cells. Under normal physiological conditions, brain cells metabolise glucose for energy. If “starved” of glucose, ketone bodies are metabolised. Mitochondrial defects in brain tumour cells obviate this metabolic flexibility resulting in glycolytic dependency. Thus, a high, low carbohydrate ketogenic diet (KD) may control tumour growth. We evaluated the expression of succinyl-CoA-3-oxoacid CoA transferase 1 (OXCT1) and D-3-hydroxybutyrate dehydrogenase 1 (BDH1), both involved in ketone body utilization and pyruvate kinase M2 (PKM2), a potential biomarker of metabolic reprogramming, in Medical University of Białystok, Białystok, Poland, 4Department of Experimental and Clinical Pathology, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland

OBJECTIVES: Previous analyses indicated that expression of ALK at the RNA level is correlated with the WNT-activated type of medulloblastoma. Therefore, we investigated if ALK expression at the protein level may serve as a useful marker for identification of this type of medulloblastoma. METHODS: Tumors from 134 patients were studied at diagnosis by immunohistochemistry using antibody ALK clone D53F #3633 (Cell Signaling). Consensus scoring was applied as the following: 0 (negative), 1 (equivocal <10%), 2 (low expression 10%-50%), 3 (moderate expression 50%-100%), 4 (high expression >100%) of positive tumor cells. Transcriptional subtypes of medulloblastoma tumors were established in all cases. Thirty infratentorial ependymal tumors were also investigated to determine if ALK protein expression may serve as a marker for differential diagnosis. RESULTS: All 19 WNT tumors were positive for ALK expression and in all but one positive reaction was present in >50% of tumor cells (score 4+). Only four ALK positive tumors were detected among the remaining 85 tumors: one tumor with a score 2+ in Group 4 and three tumors with a score 1+ (one in Group 3 and two in SHH group). Therefore, expression of ALK is strongly associated with the WNT type of disease (p=0.0001). By contrast, none of the analyzed ependymal tumors displayed positive ALK reaction. CONCLUSION: The results indicate that ALK expression at the protein level may serve as a marker for differential diagnosis for the WNT-activated type of medulloblastoma. Funded by National Science Centre, Poland (2016/21/B/NZ2/01785 and 2016/23/B/NZ2/03064).

MBRS-21. EXTRACELLULAR VESICLES FROM METASTATIC MEDULLOBLASTOMA CELL LINES CARRY MRNAS KNOWN TO BE CORRELATED WITH MBRS-22. EZH2 OVEREXPRESSION INCREASES THE ONCOGENIC POTENTIAL OF MEDULLOBLASTOMA EVS. Thus, EVs are potential diagnostic and therapeutic biomarkers for medulloblastoma patients.

Hannah J Jackson1, Franziska Linke1, Ian D Kerr2, and Beth Coyle1.
1Children’s Brain Tumour Research Centre, School of Medicine, University of Nottingham, Nottingham, UK, 2Division of Cell Signalling and Pharmacology, School of Life Sciences, University of Nottingham, Nottingham, UK

There is no curative treatment for patients with metastatic medulloblastoma, which is understood to be highly aggressive, and therefore diagnosis and treatment is a greater understanding of the mechanisms of metastasis is required, as is the development of novel diagnostic methods. Extracellular vesicles (EVs) are a heterogeneous population of nano-sized, cell derived vesicles. EVs have been shown to transfer oncogenic proteins and nucleic acid cargo to recipient cells, which modulates their activity and plays a decisive role in tumorigenesis. We hypothesised that EVs play a role in medulloblastoma metastasis. An ultra-centrifugation isolation method was optimised to selectively isolate EVs from medulloblastoma cell lines (DAOY, UW228-3, CHLA-01-MED, CHLA-01R-MED, HD-MB03 and D283). Validation was by nanoparticle tracking analysis, transmission electron microscopy and western blotting. The RNA cargo of the isolated EVs was analysed for the expression of metastasis-associated genes c-Met, ABCB1, MMP2, BSG and ITG-A9 by qRT-PCR. Immunofluorescence is also being used to localise MMP2 and EMMPRIN (BSG) encoded proteins. Our data demonstrates that medulloblastoma cells secrete two distinct populations of EVs, exosomes and microvesicles, with unique sizes and morphology and cargo. We have shown that metastatic cell lines produce significantly higher quantities of exosomes compared with non-metastatic cell lines. Finally, we have identified that candidate metastatic mRNAs; c-Met, ABCB1, MMP2, BSG and ITGA9 are present in EVs. This study provides new insights on medulloblastoma EVs. Our results indicate that mRNA of metastasis-associated genes is passed from the parent cells to EVs. Thus, EVs are potential diagnostic and therapeutic biomarkers for medulloblastoma patients.

MBRS-22. EZH2 OVEREXPRESSION INCREASES THE ONCOGENIC CHARACTER OF CEREBELLAR PROGENITORS AND BIOGRAPHS IN MICE RESULT IN TUMOURS RESEMBLING GROUP 4 MEDULLOBLASTOMA

Ismail Sola, Sujatha Venkataraman, Angela Pierce, Ilango Balakrishnan, Diane Birks, Nicholas K. Foreman, and Rajeev Vibhakar. University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA