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MDB-45. THE PTBP2 SPlicing FACTOR IS ESSENTIAL IN GROUP 3/4 MEDULLOBLASTOMA
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BACKGROUND: Medulloblastoma (MB) is a common malignant pediatric brain tumor with significant heterogeneity among its four molecular subgroups: WNT, SHH, groups 3 and 4. There is a significant rate of disease recurrence with group 3/4 MB indicating a need for novel therapeutic targets. Since transcriptomic profiling of MB has not identified novel targets, we focused on a proteomic approach. We selected 157 proteins significantly over-expressed in Group 3/4 MB and performed a targeted loss-of-function CRISPR-Cas9 screen in four MB cell lines. We identified 17 essential genes shared by at least 2 of the cell lines. The PTBP2 splicing factor was essential for all cell lines. PTBP2 is an RNA-binding protein involved in splicing regulation that plays an important role during neuronal maturation. We hypothesize that persistent expression of PTBP2 results in a differentiation arrest and preservation of proliferative potential. METHODS: We stably knocked-out PTBP2 expression in D556 and MB002 MB cells. We performed bulk RNA-seq and quantitative proteomics of these KO clones along with CLIP-seq to map PTBP2 targeted transcripts. We also performed phenotype assays, qPCR array and single-cell RNA-seq in KO clones to validate proliferation and neuronal differentiation. RESULTS: RNA-seq in PTBP2 KO MB cells revealed 2,213 differentially expressed genes predominantly involved in neuronal differentiation. These genes were cross-referenced with quantitative proteomic and eCLIP data. Using RNAseq from clinical samples, splice inclusion analysis based upon PTBP2 bound transcripts neatly segregated Group 3/4 MB from SHH and WNT. PTBP2 KO cells exhibited reduced proliferation and, upon stimulation, increased differentiated morphology compared to controls. This latter observation was further supported by qPCR array and single-cell RNA-seq. CONCLUSIONS: The results suggest that the loss of PTBP2 expression stimulates MB differentiation with a subsequent reduction of proliferation activity and nominates PTBP2 and its downstream effectors as potential therapeutic targets in group 3/4 MB.