The Role of CDK12 in Pediatric MYC-Amplified Medulloblastoma

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BACKGROUND
- Medulloblastoma is the most common type of malignant brain cancer in children, originating in the cerebellum
- MB is subdivided into four subtypes
  - WNT is the most common with the best prognosis
  - Group 3 has the worst prognosis
  - This group is MYC-amplified
- Vibhakar lab performed a functional genomic screen using CRISPR-Cas9 technology.
  - Cyclin-dependent kinase 12, CDK12, was identified as a top essential gene for Group 3 Myc-MB viability
- Using microarray data, CDK12 overexpression was identified in Group 3 MB
- Additional data also suggests that higher CDK12 expression confers worse survival rate in Group 3 MB

OBJECTIVES
- What is the role of CDK12 in Group 3 MB tumors?
- What effect do CDK12 inhibitors have on Group 3 MB cell lines?
- Does CDK12 in Group 3 MB regulate radiation sensitivity?

MATERIALS AND METHODS
- Verified group 3 MB cell lines used:
  - D458 (Med (RRID:CVCL_1161)) – MYC-amplification with CDK12 overexpression
  - D425 (Med (RRID:CVCL_1275)) – has additional TP53 mutation
  - CDK12 knock down cell lines were developed using three different Sigma Aldrich CDK12-shRNAs
  - HEK293T transfection was followed by lentiviral transduction of both D458s and D425s
- Knock down was confirmed using western blot
- CDK12 inhibitor E9 (MedChemExpress) IC50 was performed using D425s and D458s to find an IC50 of 30nm
- Neurosphere growth assay performed for KD cell lines and control cell lines treated with E9
- Western blotting using shRNA KD cell lines as well as controls
  - Probed for total RNA Pol II, c-Caspase 3/7, c-MYC, P-Rpb1-Ser2 and beta-actin

RESULTS
- Successful shRNA knock down confirmed by western blot
- Neurosphere growth assays are good indicators that group 3 MB relies heavily on CDK12

FUTURE WORK
- Further exploration of E9 mechanism via western blotting
- RT-PCR on shRNA-KD cell lines
- In vivo mouse experiments with knock downs and E9
- RNAseq data analysis for other potential therapeutic/synergistic targets

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