CDK8-Mediator complex plays positive transcriptional role in *MYC*-amplified medulloblastoma

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Background

Medulloblastoma (MB) is the most common malignant pediatric brain tumor. It is a heterogenous cancer categorized into four distinct molecular subtypes. Group 3 MB is characterized by MYC amplification and carries a poor prognosis with a 50-60% 5-year survival expectancy[1]. Group 3 MB has been a focus of therapy development in MB research given its dismal prognosis, however, it has proven difficult to find an efficacious way to target the hyper-transcriptional activity that results from MYC amplification. Currently available molecular therapies are failing to outperform the standard therapy of surgical resection, craniospinal irradiation, and adjuvant chemotherapy which has been the consistent therapy for the previous decades with little improvement. This treatment reduces quality of life for the patient and underlines the importance of the identification of novel therapeutic targets.

In this study, we investigate cyclin dependent kinase 8 (CDK8), a mediator complex-associated transcriptional regulator. CDK8-mediator complex has been well-characterized in yeast as a transcriptional regulator through interaction with the C-terminal domain of RNA Polymerase II[2]. It has been previously implicated in colorectal cancer and BCR-ABL leukemia[3,4]. CDK8 has been implicated as both a positive and negative transcriptional regulator.[5] CDK8 was identified in MYC-amplified MB as a potential actionable target by gene essentiality screening however its role in MB has yet to be defined.

Study Design

Hypothesis

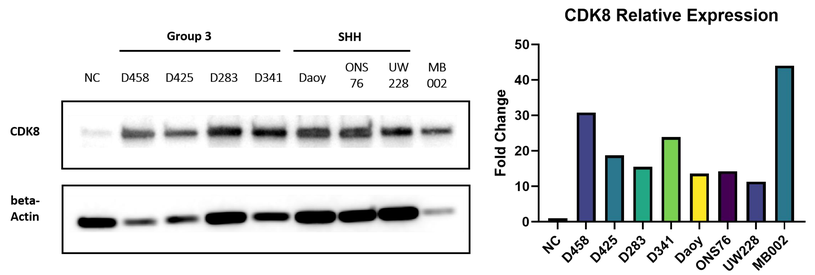
We hypothesize that CDK8 activation occurs as a direct consequence of MYC and that this increased expression enhances Myc driven transcription and chromatin activation to suppress apoptosis and promote self-renewal and radio-resistance of MB tumor stem cells.

Materials and Methods

- *In vitro* models of group 3 medulloblastoma: D458 grown in DMEM supplemented with FBS, sodium pyruvate, penicillin-streptomycin, and L-glutamine. D283 grown in DMEM supplemented with FBS, sodium pyruvate, penicillin-streptomycin, and non-essential amino acids.
- Protein expression analysis with western blotting on 4-20% SDS-PAGE. Quantification completed with ImageJ peak area analysis.
- Spheroid live cell imaging using Incucyte software with photographs taken every 24 hours. Cells grown in appropriate media with added CDK8 chemical inhibitors Senexin B (10-2000nM) and BI-1347 (0.25-50nM).
- DepMap Public 20Q4 CCLE gene expression database contains expression data derived from RNAseq data in thousands of pathologic cell lines. Expression data from excel file was retrieved, analyzed, and graphed in GraphPad Prism.

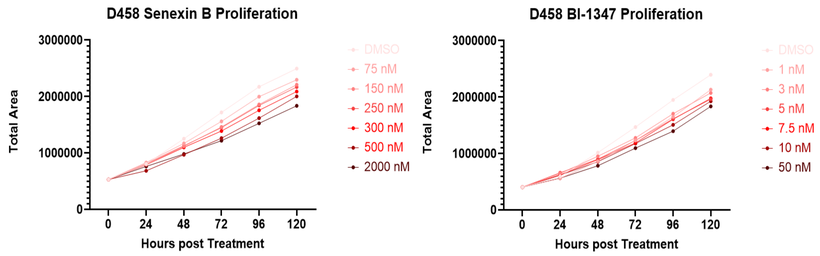
Results

Figure 1: Expression of CDK8 increased among multiple medulloblastoma subtypes



Western blotting of protein isolate from two medulloblastoma subtypes: Group 3 (D458, D425, D283, D341, MB002) and SHH (Daoy, ONS76, UW228). Quantification of CDK8 protein expression performed using ImageJ and analyzed relative to normal cerebellar expression of CDK8. Group 3 MB demonstrated marked increase in CDK8 expression ranging in all medulloblastoma lines. MYC gene expression obtained from DepMap Public gene expression database. Comparison of MYC gene expression and CDK8 amplification reveal relationship between increased CDK8 expression and increased MYC gene expression.

Figure 2: Growth reduction demonstrated with CDK8 chemical inhibition at increasing titrations



Proliferation of MYC-amplified medulloblastoma line D458 demonstrated that with increasing titrating inhibition of CDK8, growth rates decrease. Chemical inhibition of CDK8 performed with Senexin B (IC50 = 218.6 nM) and BI-1347 (IC50 = 2.591 nM).

Discussion

- The increased relative expression of CDK8 in medulloblastoma cell lines as compared to normal cerebellum support previously published findings that CDK8 is essential to MB cell survival
- Relative expression of CDK8 is noted to be higher in cell lines with strong MYC amplification (D458, D425, MB002) indicating there may be an association between transcriptional activation and CDK8 expression in group 3 medulloblastoma
- Chemical deletion of CDK8 results in growth rate reduction indicating CDK8 functions as a positive transcriptional regulator in group 3 medulloblastoma

Future Directions

- Protein analysis of chemical depletion to be conducted looking at potential downstream transcriptional targets (i.e. RNA Polymerase II and MYC) as well as mediator complex stability
- Genetic depletion studies to be run mirroring chemical depletion studies: cell proliferation experiments, and protein and RNA analysis
- Chromatin precipitation studies to be completed to elucidate the chromatin localization of CDK8-Mediator complex as well as potential interactions with MYC

References

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