

ELUCIDATING THE INTERACTION OF SHH AND BMI1 IN MEDULLOBLASTOMAS SORTED FOR CD133

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Purpose: Overexpression of Bmi1 promotes cell proliferation and is required for Hedgehog (Hh) pathway-driven tumorigenesis. The objective of this study was to determine the role of Sonic Hedgehog (Shh) and Bmi1, polycomb ring finger oncogene, in childhood medulloblastomas sorted for neural stem cell marker, CD133. Although current literature suggests that there is a correlation between Shh pathway genes and Bmi1 expression, it is unclear whether downstream effectors of Shh, such as Gli1 and Gli2, bind to and activate the Bmi1 promoter.

Methods: Microarray data was obtained from normal fetal brain and primary medulloblastoma samples, both sorted for CD133. Primary patient samples and medulloblastoma cell lines were prospectively sorted for CD133 and treated with Shh ligand and cyclopamine (Shh antagonist) to determine the expression level of Bmi1. Following treatment, RT-PCR was conducted to determine the expression levels of Bmi1 as well as Shh pathway mediators. To determine if Shh drives differentiation and/or proliferation through Bmi1, cells will be knocked down for Bmi1 and induced to differentiate. Cells will then be stained for differentiation markers like MAP2, GFAP, and TUJ1 and observed under confocal microscope. Proliferation assay will be carried out using the CyQuant assay kit. For assessment of the epigenetic regulation of Bmi1, ChIP (chromatin immunoprecipitation) will be conducted on unsorted spheres by immunoprecipitating with Gli1/Gli2 antibody and analyzing the presence of Bmi1 promoter through qPCR.

Results: Our previous data have shown that Bmi1 is upregulated in the CD133- population. Interestingly, our microarray data indicate that several Shh pathway genes are also significantly upregulated in the CD133- population compared with its positive counterpart. We confirmed these findings with RTPCR by demonstrating that Shh and Bmi1 are upregulated in the CD133- population in medulloblastomas. Furthermore, medulloblastoma samples expressed different Shh and Bmi1 pathway genes than the normal fetal brains. Preliminary data from the Shh treated samples indicate that Bmi1 expression positively correlates with increasing Shh ligand concentrations. Moreover, we observed that Shh pathway genes, such as Gli1 and Gli2, correlate strongly with Bmi1 expression.

Conclusion: This report is the first indication that Shh and Bmi1 is upregulated in the CD133- population in medulloblastomas. More study is needed to determine their role in this population. We also demonstrate a clear correlation between Shh pathway genes and Bmi1 expression, suggesting an epigenetic regulatory mechanism and providing potential chemotherapeutic targets.

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