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The Role of RASSF5 on Cell Growth and Hippo Signaling in Rhabdomyosarcoma

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Introduction: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood. Dysregulation of the Hippo pathway, a signaling cascade that regulates many biological processes, is associated with many human cancers. The goal of my project was to delete RASSF5, a regulator of the Hippo pathway, in RMS cells utilizing CRISPR/Cas9 and then to evaluate how the absence of RASSF5 affects RMS cell growth and Hippo signaling, with and without DNMTi Tx.

Methods: A lipofectamine transfection was performed in two different RMS cell lines, Rh30 & RD, in which two different CRIPSR/Cas9 vectors with RASSF5 guide RNA were introduced. Then IncuCyte growth assays, western blot and qPCR were performed

Results: The IncuCyte growth curve for one of the RASSF5 CRISPR Rh30 cell lines, Sg1, revealed a faster rate of cell growth compared to the control Rh30s. Also, when treated with SGI110 Tx, there is reduction of drug induced growth inhibition of Sg1 Rh30 cells compared to controls. When looking at the protein level, although Cas9 expression was observed, there was no baseline reduction in RASSF5.

Discussion: While some of this data suggests that we have less activation of the Hippo pathway, which would result from a reduction in RASSF5, other data implies that RASSF5 was not deleted entirely. Further research is needed to elucidate RASSF5's role in both RMS and the altered Hippo pathway in RMS.