Title: Investigating the Loss of H3K27me3 via PRC2 as a Cause of Genome Instability in MPNST

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Malignant Peripheral Nerve Sheath Tumors (MPNSTs) exhibit extensive genome instability with complex chromosomal aberrations, including gains, losses, and rearrangements of chromosomal regions leading to gene dosage imbalances. In about 80% of MPNST cases, Polycomb Repressive Complex 2 (PRC2) function is lost via biallelic mutations in SUZ12 or EED, which results in a global loss of H3K27me3, a repressive chromatin mark. It has been found that H3K27me3 is required for Topoisomerase IIα (Topo II) to properly decatenate sister chromatids during mitosis. Topo II is needed to release supercoils and decatenate DNA for high fidelity chromosome segregation. A loss of either the chromatin tether domain of Topo II or ablation of H3K27me3 results in error prone anaphases including chromosome bridges, lagging chromosomes, and ultra-fine DNA bridges. The purpose of this study is to determine if the genome instability observed in PRC2 deficient MPNSTs might be caused by the inability of Topo II to decatenate sister chromatids via H3K27me3. We have found that immortalized human NF1-deficient Schwann cells with the loss of PRC2 have more aberrant anaphases and ultra-fine DNA bridges than controls. In addition, we have performed a drug screen with Topo II, Aurora B, and Haspin Kinase inhibitors to investigate if further disruption of the metaphase Topo II checkpoint exhibits synthetic lethality with PRC2 loss in immortalized human Schwann cells. This drug study has revealed that the loss of PRC2 causes the cells to become more sensitive to all three classes of drugs tested. To examine the mechanism of cell death after drug treatment we are performing flow cytometry to analyze the phases of the cell cycle and apoptosis. In summary, we have found evidence that the inability of Topo II to target chromatin due to the loss of H3K27me3 after PRC2 inactivation could be related to the genome instability observed in MPNST. Completion of this study will determine if the loss of PRC2 is a factor in the acquisition of aneuploid genomes in MPNST and provide therapeutic rationale for drugs targeting the metaphase Topo II checkpoint.