Gene Regulation by miRNAs and PRC2 in Glioblastoma Multiforme

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Introduction: Gene regulation occurs in a variety of ways, including via transcriptional and post-transcriptional pathways. Two major players in these pathways are polycomb repressive complex 2 (PRC2) and micro-RNAs (miRNA) [1,2]. PRC2 and miRNAs are both important in glioblastoma multiforme (GBM), the most common and lethal primary adult brain cancer[3,4]. PRC2 is an epigenetic regulator that works transcriptionally while miRNAs are short single-stranded RNA molecules that work post-transcriptionally. Our preliminary data suggests that PRC2 activates several genes involved in interferon response pathway by repressing miRNAs that target them (Unpublished). How this regulatory mechanism impacts interferon response and its role in cancer is yet unknown.

Materials and Methods: To check if the interferon pathway genes we found to be activated by PRC2 in T98G GBM cell line are also activated in primary tumors, we analyzed pairwise correlation between the expression of PRC2 components-EZH2 and SUZ12, and EZH2-activated interferon genes using data from the Cancer Genome Atlas (TCGA). To further determine if PRC2 activated the interferon pathway genes by repressing the same set of identified miRNAs that we identified in T98G cells, we tested for negative correlation between PRC2 components and EZH2-repressed miRNAs. Lastly, we tested the role of PRC2 on interferon response by checking for differences in ISG induction in cells lacking PRC2 components. EZH2-/-, SUZ12-/- and wild type GBM cell lines were treated with interferon-gamma for 48 hours at a concentration of 30ng/mL. ISG induction levels were then measured through qRTPCR and immunoblotting.

Results and Discussion: Our analysis of TCGA mRNA-seq datasets from low grade glioma samples showed a higher fraction of interferon pathway genes (58% of annotated interferon pathway genes) to be positively correlated with PRC2 component expression. We also found a majority of the EZH2-repressed miRNAs (68% of the EZH2-repressed miRNAs) to be negatively correlated with PRC2. We found that knockout of EZH2 and SUZ12 impacts ISG induction in response to interferon-gamma treatment. Our qRT-PCR data showed that loss of EZH2 led to a potential decrease in MX1 induction but an increase in MX2 and no changes in IL-6 induction. Western Blot analysis showed decreases in pSTAT1 levels in EZH2 -/- cells when compared to WT while SUZ12 -/- showed even lower amounts.

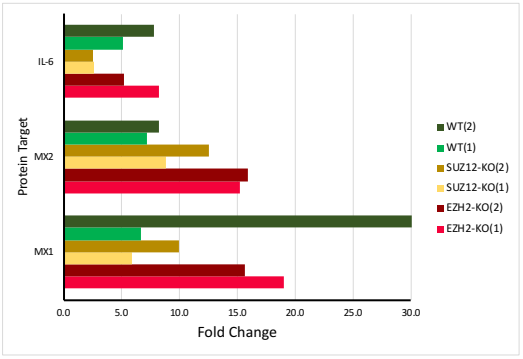

Figure 1. Fold change of induction of ISGs between treatment and non-treatment cells measured through qRTPCR.

Conclusions: Our analysis of TCGA data suggests that the regulatory pathway involving indirect activation of IFN pathway genes by PRC2 through repression of miRNAs is also likely to occur in solid tumor samples. Effect of PRC2 loss on ISG induction based on qRTPCR, so far appears to be inconclusive and highly gene-specific. However, activation of STAT1 through phosphorylation in response to IFN treatment showed significant reduction in cells lacking PRC2. This suggests that PRC2 promotes interferon response possibly through activation of IFN pathways genes via miRNAs.

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