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Mutant IDH Alleles Cause Methylation Changes in the Human Genome Which Alter Gene Expression in Genes with Differentially Methylated Gene Promoters

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Acute myeloid leukemia (AML) is an aggressive form of cancer that occurs in hematopoietic stem cells. Previous work demonstrated that AML tumors contain many recurrent mutations. Some of these mutations occur in genes that are critical for epigenetic regulation, such as *DNMT3A*, *TET2*, or *IDH1* and *IDH2* (collectively referred to as *IDH*). Mutations in *IDH* occur in about 20% of AML samples. Mutant IDH enzyme develops neomorphic function; it produces the oncometabolite 2-hydroxyglutarate (2HG) instead of alpha-ketoglutarate. 2HG has been associated with DNA hypermethylation and increased histone methylation in AML. Both epigenetic changes alter the chromatin state, which can affect gene expression. Our lab hypothesizes that expression of mutant *IDH2* alleles increases the amount and type of methylation at genomic loci that can affect the transcription of genes with promoters located in differentially methylated regions (DMRs) of *IDH* mutant and wild type AML. To assess mutant *IDH2*-specific dysregulation, our lab developed a line of transgenic H9 human embryonic stem (hES) cells with a doxycycline (DOX)-inducible $IDH2^{R140Q}$ mutant allele. We optimized DOX treatment for both expression of the mutant allele and cell viability by manual counting with a hemocytometer and then confirmed by mass spectrometry that mutant IDH2 produces 2HG. As for changes in methylation, we demonstrated by Western blotting that expression of mutant *IDH2* increases overall trimethylated H3K9, a histone marker of gene silencing. We are currently preparing transgenic hES cell DNA for TrueMethyl bisulfite and oxidative bisulfite sequencing to quantify and locate DNA methylation. To understand differences in expression due to methylation, we took a subset of 871 unique gene promoters located in the DMRs of DNA. Using RNA-Seq data, we identified nineteen genes with dysregulation beyond what would be predicted. PANTHER Gene List Analysis of the 871 unique genes identified consistent themes for protein and nucleic acid binding as well as hydrolase and transferase activity.