HIF1-ALPHA Promotes ID2 Expression through Novel HRE Sites in the ID2 Promoter

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Inhibitor of DNA binding 2, or Id2, is a protein that is required for hematopoietic stem cell (HSC) maintenance and self-renewal capacity. A positive feedback loop has been identified involving ID2 and Hypoxia Inducible Factor 1α (HIF1α), where expression of either protein increases the others' expression. Both HIF1α and ID2 has been shown to be essential for HSC quiescence. The transcriptional activity of HIF1α is directed by hypoxia response elements (HRE), where HIF1α binds directly to HRE sites. Two HRE sites were newly identified in the ID2's promoter, thus it was hypothesized that these sites were required for the HIF1α mediated increase in ID2 expression. Indeed, it was found that the HIF1α expression promoted ID2 transcription. The sites were then mutated to determine if HIF1α acted through these two specific HRE's in the ID2 promoter. Mutated HRE reporter plasmids pGL 4.10 and pGL 4.10 Id2pro were transfected into HEK 293 cells with empty vector pcDNA3.1 or pcDNA3.1 with HIF1α. A luciferase reporter assay quantified the activity of HIF1α on the Id2 promoter. HRE 1 and HRE 2 mutated plasmids displayed lower levels of luciferase activity than the unmutated ID2 promoter control and comparable levels to the empty vector control pGL 4.10 in the presence of HIF1α. The findings show that HIF1α regulates ID2 expression through novel HRE sites. Importantly, these studies promote further investigation of the ID2-HIF1α regulatory loop. Future studies will investigate HIF1α gain of function and loss of function in HSCs with respect to ID2 expression.