GLYCOGEN SYNTHASE KINASE 3β (GSK3β) TARGETS HYPOXIA INDUCIBLE FACTOR-1α (HIF-1α) FOR PROTEASOMAL DEGRADATION DURING HYPOXIA

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Hypoxia Inducible Factor-1α (HIF-1α) mediates expression of genes associated with endothelial cell-mediated angiogenesis and is associated with poor outcomes in a variety of cancers. In normoxia, HIF-1α is ubiquitinated and degraded through interactions with the E3 ubiqutin ligase, von Hippel-Lindau (vHL); however, little is known about the regulation of HIF-1α in hypoxic conditions. FBW7, an E3 ubiquitin ligase, has been shown to interact with several transcription factors including those phosphorylated by Glycogen Synthase Kinase 3β (GSK3β). This study tested the hypothesis that phosphorylation of HIF-1α by GSK3β increases the FBW7 mediated ubiquitin-dependent degradation of HIF-1α, thereby resulting in suppression of the hypoxia-mediated angiogenic response. Using immunoblot, expressed β and γ isoforms of FBW7 decreased HIF-1α stabilization and co-immunoprecipitation demonstrated that HIF-1α interacts with FBW7. Furthermore, in hypoxia knockdown of FBW7 using siRNA resulted in increased HIF-1α expression. HIF-1α protein and VEGF transcript levels in hypoxia were increased when GSK3β activity was inhibited and were reduced by transient expression of constitutively active GSK3β (GSK3S9A). Additionally, expression of GSK3S9A increased HIF-1α ubiquitination. Stable SKOV-3 ovarian cancer cell lines were created that express GSK3S9A or shRNA against GSK3β (shGSK3). Conditioned media from the cell lines exposed to hypoxia stimulated endothelial cell tube formation. Tube maturation was significantly increased by shGSK3 media, suggesting that GSK3β exerts an inhibitory effect on hypoxia-mediated angiogenesis. These data suggest a new mechanism for negative regulation of HIF-1α during hypoxia that utilizes phosphorylation to target HIF-1α for ubiquitination and proteosomal degradation. Results of this study better define the signaling pathways necessary for HIF-1α-mediated signaling and may identify new targets that mediate angiogenesis in disease.