DIFFERENTIAL FUNCTION AND REGULATION OF THE HYPOXIA INDUCIBLE FACTORS IN THE RAT PHEOCHROMOCYTOMA CELL LINE PC12

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THESIS SUMMARY

Responses to hypoxia in mammals include enhanced erythropoiesis, angiogenesis and expression of enzymes responsible for glucose transport and glycolysis. Hypoxia inducible factor 1α (HIF-1α) and HIF-2α are involved in many of the molecular and physiological responses to low oxygen levels. Both are members of the basic Helix-Loop-Helix/Per-ARNT-Sim (bHLH/PAS) protein family and form DNA binding heterodimers with the aryl hydrocarbon receptor nuclear translocator (ARNT), another bHLH/PAS protein. In normoxia, HIF-1α and HIF-2α are degraded in the cytoplasm by a mechanism involving oxygen dependent hydroxylation of conserved prolines in their respective oxygen dependent degradation domains, which permits the recruitment of the von Hippel-Lindau E3 ligase and targeting of the protein for destruction via the ubiquitin proteasome pathway. Also, oxygen dependent hydroxylation of an asparagine in the carboxy terminal transactivation domain of HIF-1α and HIF-2α results in the repression of their transcriptional activity. During hypoxia, HIF-1α and HIF-2α undergo protein stabilisation, derepression and translocation from the cytoplasm to the nucleus, where they dimerise with the constitutively nuclear ARNT to bind to hypoxia response elements (HREs) and induce transcription of HRE associated genes.

Despite the biochemical similarities between the hypoxic regulation of HIF-1α and HIF-2α, gene targeting experiments show major differences in developmental abnormalities between HIF-1α$^{-/-}$ and HIF-2α$^{-/-}$ mice and thus suggest distinct physiological roles and target gene specificities. A number of HIF-1α specific target genes have been documented, but there are currently none that have been conclusively identified for HIF-2α. The aim of this project was to determine specific gene targets and differential regulation of HIF-1α and HIF-2α in the rat pheochromocytoma cell line PC12.

Both HIF-1α and HIF-2α proteins are known to accumulate during hypoxia in PC12s. Furthermore, PC12s display a hypoxically inducible catecholamine synthesis and release pathway, a disruption of which is believed to contribute to the embryonic lethal phenotype of HIF-2α null mice. Therefore, monoclonal lines were established in which either HIF-1α or HIF-2α could be selectively stabilised and activated. Northern analysis of these lines demonstrated that the mRNA levels of many known targets of HIF-1α are induced by

HIF-1α but not HIF-2α. This suggests that HIF-2α may have its own novel targets. Furthermore, DNA microarray analysis was employed using these inducible cell lines and has uncovered a number of novel putative HIF-2α target genes which may relate to the physiological role of this transcription factor.

A surprising feature of these PC12 cell line derivatives is that the selective upregulation of HIF-1α does not induce HIF-2α, and *vice versa*. This suggests uncharacterised differences in their degradation mechanisms. For HIF-1α, it was demonstrated that destruction is initiated by prolyl-4-hydroxylation, albeit without a requirement for 2-oxoglutarate, which is considered a cosubstrate of the HIF-α prolyl-4-hydroxylases. In contrast, it is shown that HIF-2α levels are not mediated by prolyl-4-hydroxylation. Rather, preliminary data suggest that lysine acetylation is involved in regulating HIF-2α protein stability, but by a mechanism which is independent of the function of the only known HIF-α lysyl acetylase, ARD1. This study clearly shows the differential regulation of endogenous HIF-1α and HIF-2α proteins in the model PC12 cell line and suggests that similar regulation *in vivo* may contribute to the distinct physiological roles of these factors.