CHARACTERISING THE DIFFERENTIAL REGULATION OF HYPOXIA-INDUCIBLE FACTOR 1α AND 2α

Submitted for the degree of Doctor of Philosophy

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In animals, the transcriptional responses to hypoxia primarily aim to increase oxygen delivery to cells and facilitate anaerobic glycolysis, in addition to other processes including cell matrix assembly, growth and apoptosis. The hypoxia-inducible transcription factors, HIF-1α and HIF-2α, are key mediators of these responses. The HIF-αs themselves are primarily regulated through the dual control mechanisms of stabilisation and transactivation, mediated by prolyl and asparaginyl hydroxylation. Despite high conservation in domain architecture and amino acid sequence, in addition to similarities in DNA binding and hydroxylation-dependent control, there are distinct biochemical and physiological differences between HIF-1α and HIF-2α, best demonstrated by the non-redundant nature of the HIF-α knockout mice. Very few mechanistic differences between HIF-1α and HIF-2α however are known, which cannot explain this non-redundancy. The aim of this PhD thesis is therefore to determine novel mechanisms regulating HIF-1α and HIF-2α that may underlie these differential properties.

To achieve this aim, a yeast two-hybrid screen was initially employed using a region of HIF-2α possessing the highest sequence divergence with HIF-1α. The NF-κB Essential Modulator, NEMO, was identified as a HIF-2α, but not HIF-1α, interacting protein which was subsequently confirmed in mammalian cells by various pulldown and immunoprecipitation methods. Furthermore, NEMO specifically increased HIF-2α activity at normoxia, both when overexpressed and at endogenous levels, via a mechanism consistent with the enhanced recruitment of the CBP / p300 coactivators.

An additional strategy to analyse differential HIF-1α / 2α regulation was to compare their O_2-regulated stabilisation and transactivation across a number of mammalian cell lines. Although generally, the HIF-α proteins were stabilised and transcriptionally activated over a similar O_2 range, interesting cell-specific differences were apparent, both when comparing HIF-1α and HIF-2α and when comparing different cell-lines to each other. For example, iron chelation does not stabilise HIF-2α protein, although HIF-2α transactivation, and HIF-1α stabilisation and transactivation are strongly increased. Cell lines also display large differences in the O_2-responsiveness of HIF-α transcriptional activation.

In vitro, the HIF-2α C-terminal transactivation domain (C-TAD) is less efficiently hydroxylated by FIH-1 than HIF-1α. This would be expected to increase CBP / p300 recruitment which parallels the increased activity of HIF-2α C-TAD compared with HIF-1α in cells. Interestingly, the residue immediately C-terminal to the hydroxy-asparagine differs between HIF-1α and HIF-2α though is then fully conserved across species. *In vitro* hydroxylation and cell-based reporter assays were employed to identify this amino acid as an important, though not sole, contributing factor specifying the inherent differences in FIH-1 mediated C-terminal hydroxylation.

Work comprising this thesis identifies NEMO as a HIF-2α specific interacting and regulatory factor, in addition to uncovering further insights into the cell-specific nature of HIF-α regulation. This complements an emerging body of evidence demonstrating that multiple factors appear responsible for the differential regulation of HIF-1α and HIF-2α that underlie their non-redundant functions.