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

Submitted for the degree of Doctor of Philosophy

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TABLE OF CONTENTS

TABLE OF CONTENTS .............................................................. iv
THESIS SUMMARY .............................................................. vii
CANDIDATE'S DECLARATION ................................................... ix
ACKNOWLEDGEMENTS .......................................................... x
CHAPTER 1 - INTRODUCTION .................................................. 1
  1.1 HIF discovery and classification ........................................ 3
  1.2 Additional HIF-α proteins ................................................ 8
  1.3 HIF-α splice variants ...................................................... 9
  1.4 HIF-α stabilisation and hydroxylation ................................... 12
  1.5 HIF-1α transcriptional activation ......................................... 21
  1.6 Alternate mechanisms of HIF-α regulation ............................. 25
  1.7 HIF target genes ........................................................... 27
  1.8 HIF and disease ............................................................ 28
  1.9 Differential functions of HIF-1α and HIF-2α ........................... 32
  1.10 Aims and approach ....................................................... 34

CHAPTER 2 - MATERIALS AND METHODS .................................... 37
  2.1. ABBREVIATIONS .......................................................... 39
  2.2. MATERIALS .................................................................. 41
    2.2.1 GENERAL MATERIALS .................................................. 41
    2.2.2 CHEMICALS AND REAGENTS ........................................ 41
    2.2.3 COMMERCIAL KITS .................................................... 42
    2.2.4 ENZYMES ............................................................... 42
    2.2.5 RADIOCHEMICALS ....................................................... 43
    2.2.6 ANTIBODIES ............................................................ 43
      2.2.6.1 PRIMARY ANTIBODIES ............................................. 43
      2.2.6.2 SECONDARY ANTIBODIES ....................................... 43
    2.2.7 BACTERIAL STRAINS .................................................. 44
    2.2.8 YEAST TWO-HYBRID STRAIN ......................................... 44
    2.2.9 TISSUE CULTURE CELL LINES ...................................... 44
    2.2.10 BUFFERS AND SOLUTIONS .......................................... 45
      2.2.10.1 GENERAL SOLUTIONS ............................................. 45
      2.2.10.2 BACTERIAL GROWTH MEDIA ................................... 47
    2.2.11 PLASMIDS ............................................................. 47
      2.2.11.1 REPORTER PLASMIDS (obtained from elsewhere) .......... 47
      2.2.11.2 CLONING AND EXPRESSION PLASMIDS (obtained from elsewhere) .......... 47
      2.2.11.3 CLONING AND EXPRESSION VECTORS (cloned myself) ....... 48
    2.2.12 OLIGONUCLEOTIDES ................................................ 51
      2.2.12.1 - PRIMERS FOR CLONING AND SEQUENCING .................. 51
      2.2.12.2 PRIMERS FOR SITE-DIRECTED MUTAGENESIS ................ 53
  2.3 ELECTRONIC RESOURCES ................................................. 53
  2.4 METHODS .................................................................. 53
    2.4.1 YEAST TWO-HYBRID .................................................. 53
    2.4.2 DNA MANIPULATION METHODS ...................................... 54
      2.4.2.1 AGAROSE / TBE GEL ELECTROPHORESIS ...................... 54
      2.4.2.2 RESTRICTION DIGESTION OF PLASMID DNA ............... 54
3.2.8 TARA
3.2.1 PREPARATION OF COMPETENT BACTERIA
3.2.2 TRANSFORMATIONS
3.2.3 AMMONIUM ACETATE DNA PURIFICATION (Midiprep)
3.2.4 AMMONIUM ACETATE DNA PURIFICATION (Miniprep)
3.2.5 GEM-T CLONING
3.2.6 A-TAILING
3.2.7 PREPARATION OF COMPETENT BACTERIA
3.2.8 TRANSFORMATIONS
3.2.9 ALKALINE LYSIS DNA PURIFICATION (Miniprep)
3.2.10 AMMONIUM ACETATE DNA PURIFICATION (Midiprep)
3.2.11 GEL PURIFICATION OF PCR AMPLIFIED OR DIGESTED DNA

2.4.3 PCR PROTOCOLS
2.4.3.1 AMPLIFICATION OF DNA VIA PCR WITH PFU POLYMERASE
2.4.3.2 AMPLIFICATION OF DNA VIA PCR WITH TAU POLYMERASE
2.4.3.3 DNA SEQUENCING
2.4.3.4 QUICKCHANGE SITE-DIRECTED MUTAGENESIS
2.4.4 PROTEIN MANIPULATION
2.4.4.1 BRADFORD PROTEIN CONCENTRATION ASSAY
2.4.4.2 WHOLE CELL PROTEIN EXTRACTION
2.4.4.3 DENATURING PAGE FOR PROTEIN SEPARATION
2.4.4.4 WESTERN BLOTTING
2.4.4.5 GST PULLDOWN
2.4.4.6 IMMUNOPRECIPITATION
2.4.4.7 2D GEL ELECTROPHORESIS
2.4.4.8 PULSE CHASES
2.4.4.9 In vitro TRANSCRIPTION / TRANSLATION
2.4.4.10 IMMUNOFLOUORESCENCE

2.4.5 CELL CULTURE
2.4.5.1 CELL CULTURE AND REPORTER ASSAYS
2.4.5.2 DUAL-LUCIFERASE REPORTER ASSAYS
2.4.5.3 HYPOXIC TREATMENT OF CELLS
2.4.6 HYDROXYLATION (CO2 CAPTURE) ASSAY
2.4.6.1 EXPRESSION AND PURIFICATION OF MBP-FIH-1
2.4.6.2 EXPRESSION AND PURIFICATION OF Trx-6His-HIFα
2.4.6.3 EXPRESSION AND PURIFICATION OF GST-NEMO
2.4.6.4 PERFORMING HYDROXYLATION ASSAYS

CHAPTER 3 - CHARACTERISING HIF-2α SPECIFIC PROTEIN INTERACTIONS
3.1 BACKGROUND
3.2 RESULTS
3.2.1 Identifying candidate HIF-2α interacting proteins
3.2.2 Full-length TARA does not interact with HIF-2α
3.2.3 NEMO and the NF-κB pathway
3.2.4 NEMO specifically interacts with HIF-2α
3.2.5 NEMO dose dependently increases HIF-2α activity
3.2.6 HIF does not affect NF-κB activation
3.2.7 Endogenous NEMO increases HIF-2α activity
3.2.8 Characterising the mechanism of HIF-2α regulation by NEMO
3.2.9 NEMO does not specifically alter HIF-2α C-TAD hydroxylation
3.2.10 NEMO does not alter the subcellular localisation of HIF-2α
3.2.11 NEMO does not facilitate HIF-2α post-translational modification
3.2.12 NEMO increases HIF-2α transactivation, not protein stability, in a C-TAD independent manner .......................................................... 119
3.2.13 NEMO increases HIF-2α activity via p300 recruitment ............................. 126

3.3 DISCUSSION .................................................................................................................. 133

CHAPTER 4 - REGULATED HIF-α STABILISATION AND TRANSACTIVATION 141
4.1 BACKGROUND ............................................................................................................... 143
4.2 RESULTS ....................................................................................................................... 147
  4.2.1 FIH-1 expression and stability .................................................................................. 147
  4.2.2 FIH-1 knockdown increases HIF-α C-TAD activity ................................................ 147
  4.2.3 Stabilisation of HIF-1α versus HIF-2α in mammalian cell lines ......................... 151
  4.2.4 Hypoxia increases C-TAD activity without increasing protein levels .................. 167
  4.2.5 HIF-1α and HIF-2α C-TADs have the same half life ........................................... 167
4.3 DISCUSSION ................................................................................................................... 171

4.4 A MECHANISM OF DIFFERENTIAL HIF-1α AND HIF-2α ASPARAGINYL HYDROXYLATION .......................................................... 173
  4.4.1 INTRODUCTION ..................................................................................................... 173
4.5 RESULTS ......................................................................................................................... 177
  4.5.1 HIF-1α A804V and HIF-2α V848A substitution is sufficient to swap hydroxylation
        efficiency in vitro ........................................................................................................ 177

CHAPTER 5 - DISCUSSION .................................................................................................. 187

CHAPTER 6 - REFERENCES ................................................................................................ 195
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 O2-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 O2 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 O2-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.