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Free Radical and Radiation Biology Program
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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Hypoxia-Inducible Factor

by

Suwimol Jetawattana

Department of Radiation Oncology

Free Radical and Radiation Biology

The University of Iowa

Iowa City, IA 52242-1181

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Hypoxia-Inducible Factor (HIF)

- The major actor in the cellular response to low O_2 (hypoxia) by regulates the expression of many molecules that are involved in glucose transport, glycolysis, erythropoisis, iron transport and angiogenesis

- First discovered during the studies of human erythropoitin enhancer in response to hypoxia

Semenza GL, Wang GL. (1992) Mol Cell Biol. 12(12):5447-5454.

Basic Characteristics

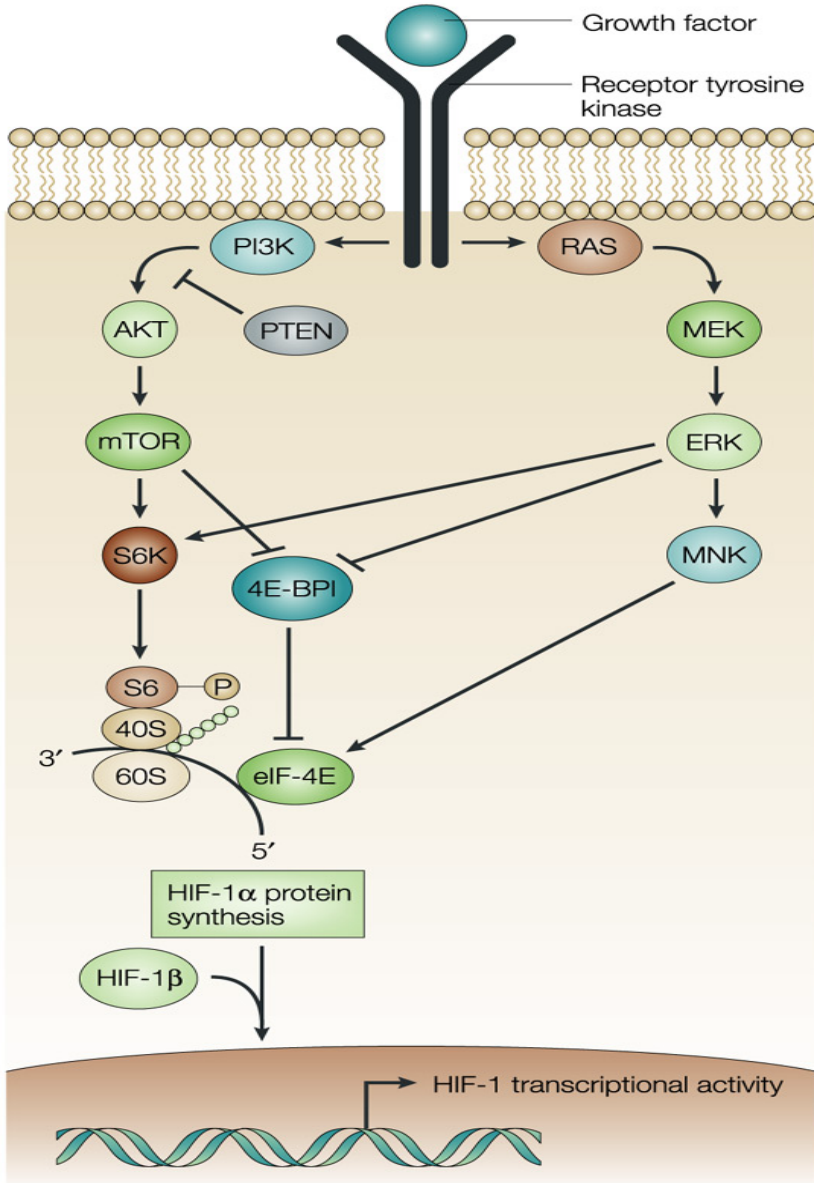
- Transcription factor
- Heterodimeric DNA binding protein
  - Constitutive nuclear subunit (HIF-1β) (also called aryl hydrocarbon receptor nuclear translocator, ARNT) : 91-94 kDa
  - Hypoxia subunit (HIF-1α) : 120 kDa

Wang GL, Semenza GL. (1995) J Biol Chem. 270:1230-1237.

Regulation of HIF-1α protein synthesis

- Growth-factor binding to a cognate receptor tyrosine kinase activates the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways.

- PI3K activates the downstream serine/threonine kinases AKT (also known as protein kinase B (PKB) and mammalian target of rapamycin (mTOR).

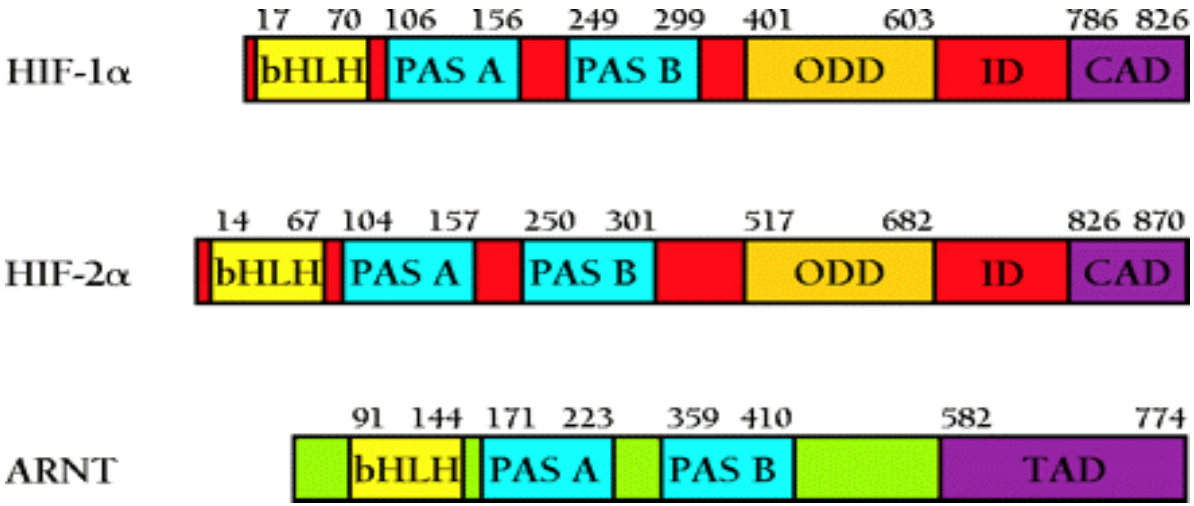


- In the MAPK pathway, the extracellular-signal-regulated kinase (ERK) is activated by the upstream MAP/ERK kinase (MEK). ERK, in turn, activates MNK. ERK and mTOR phosphorylate p70 S6 kinase (S6K) — which, in turn, phosphorylates the ribosomal S6 protein — and the eukaryotic translation initiation factor 4E (eIF-4E) binding protein (4E-BP1).

- Binding of 4E-BP1 to eIF-4E inactivates the latter, inhibiting cap-dependent mRNA translation. Phosphorylation of 4E-BP1 prevents its binding to eIF-4E. MNK phosphorylates eIF-4E and stimulates its activity directly. The effect of growth-factor signalling is an increase in the rate at which a subset of mRNAs within the cell (including HIF-1 mRNA) are translated into protein.

Semenza GL. (2003) Nature Reviews Cancer (3): 721-732.

Schematic representation of human HIF-1α, HIF-2α, and ARNT.



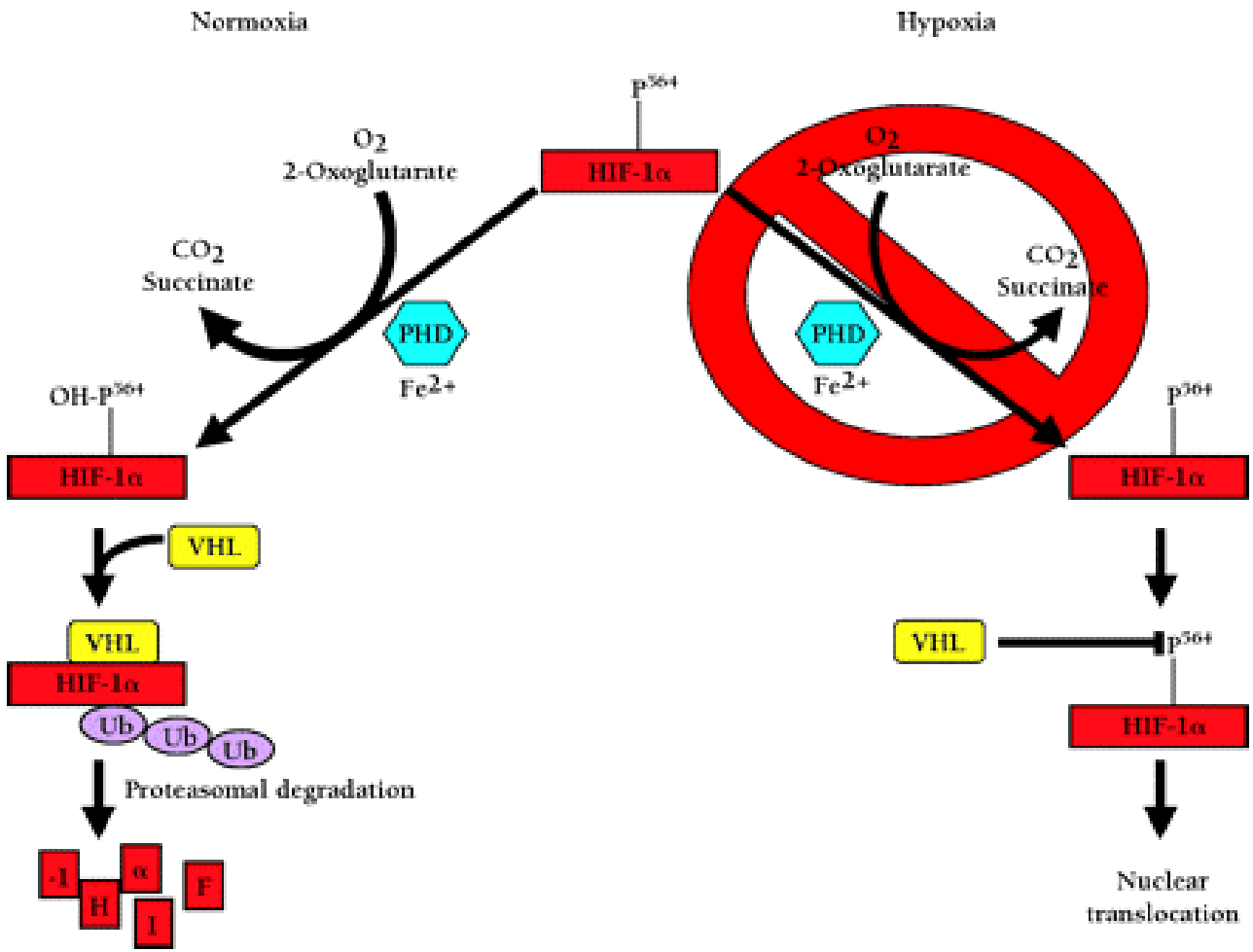
- HIF-1α, HIF-2α, and ARNT are basic-Helix-Loop-Helix-Per-ARNT-Sim (bHLH-PAS) proteins that contain an N-terminal bHLH domain and two PAS domains that are required for dimerization and DNA binding.

- HIF-1α and HIF-2α also contain an oxygen-dependent degradation domain (ODD) that mediates oxygen-regulated stability, and a C-terminal transactivation domain (CAD) whose transcriptional repression in normoxia is controlled by the inhibitory domain (ID).

- ARNT has a transactivation domain (TAD) that serves no function in the context of HIF-α activity. Amino acid numbers for each domain are indicated.

Fedele AO, Whitelaw ML, Peet DJ. (2002) Molecular Interventions 2:229-243.

Oxygen-dependent degradation of HIF-1α's



Fedele AO, Whitelaw ML. Peet DJ. (2002) Molecular Interventions. 2:229-243.

Normoxic conditions and the degradation of HIF-α

- HIF-α is constantly being made
- If O_2 is adequate, HIF-α is marked for degradation by hydroxylation reaction
- HIF-1α is hydroxylated by O_2-, 2-oxoglutarate-, ascorbate-, and Fe^{2+}-dependent prolyl-4-hydroxylase (PHDs) at proline residues 402 and 564.
- The hydroxylations target HIF-1α for binding with Von Hippel-Lindau protein (VHL) subunit of the Ubiquitine E3 ligase that mediates its rapid proteolysis by the 26S proteasome in cytoplasm.
- Activity of these PHDs is dependent on the presence of regular concentration of oxygen (O_2) and Fe^{2+}.

Tanimoto K, et al. (2000) Eur. Mol. Biol. Org. J. 19:4298–4309.

Normoxic conditions and the degradation of HIF-α

- Binding to VHL are also enhanced by the acetylation of the Lys532 by the ARD1 acetyltransferase, suggesting that the acetylation of HIF-1α by ARD1 is critical to proteasomal degradation.
- Therefore, the role of ARD1 in the acetylation of HIF-1α provides a key regulatory mechanism underlying HIF-1α stability.

Jeong JW, et al. (2002) Cell. 111:709–720.

- Iron chelators (ciclopirox olamine, CPX) and $CoCl_2$ prevent HIF-1α degradation

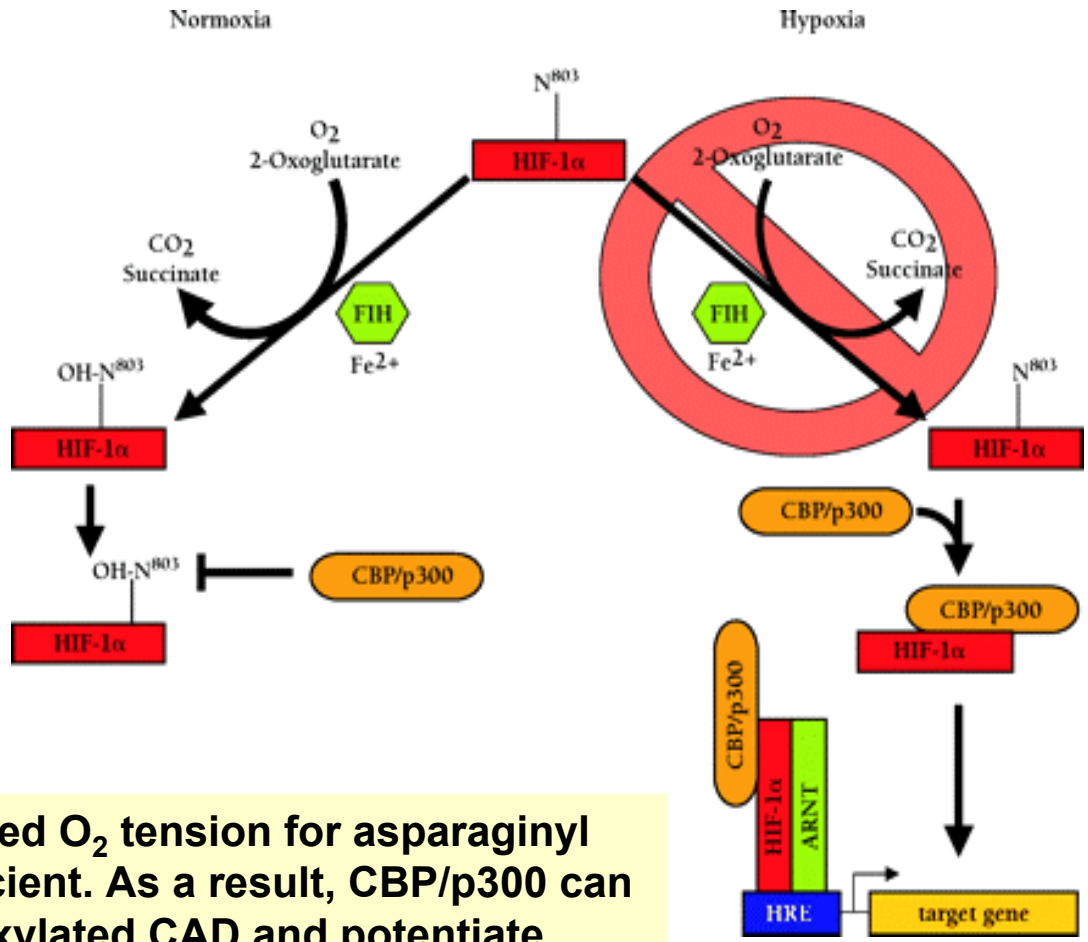
Baby SM, et al. (2003) Cell Biol. 120:343–352.

Yang DI, et al. (2004) Br J Pharmacol. 141:988–996.

Oxygen-dependent transcriptional inactivation of HIF-1α

- In the presence of oxygen, β-hydroxylation of the C-terminal Asn803 located in the transactivation domain of HIF-1α by the hydroxylase factor inhibiting HIF-1 (FIH-1) factor, inhibits its transcriptional activity by preventing the binding of the co-activator p300

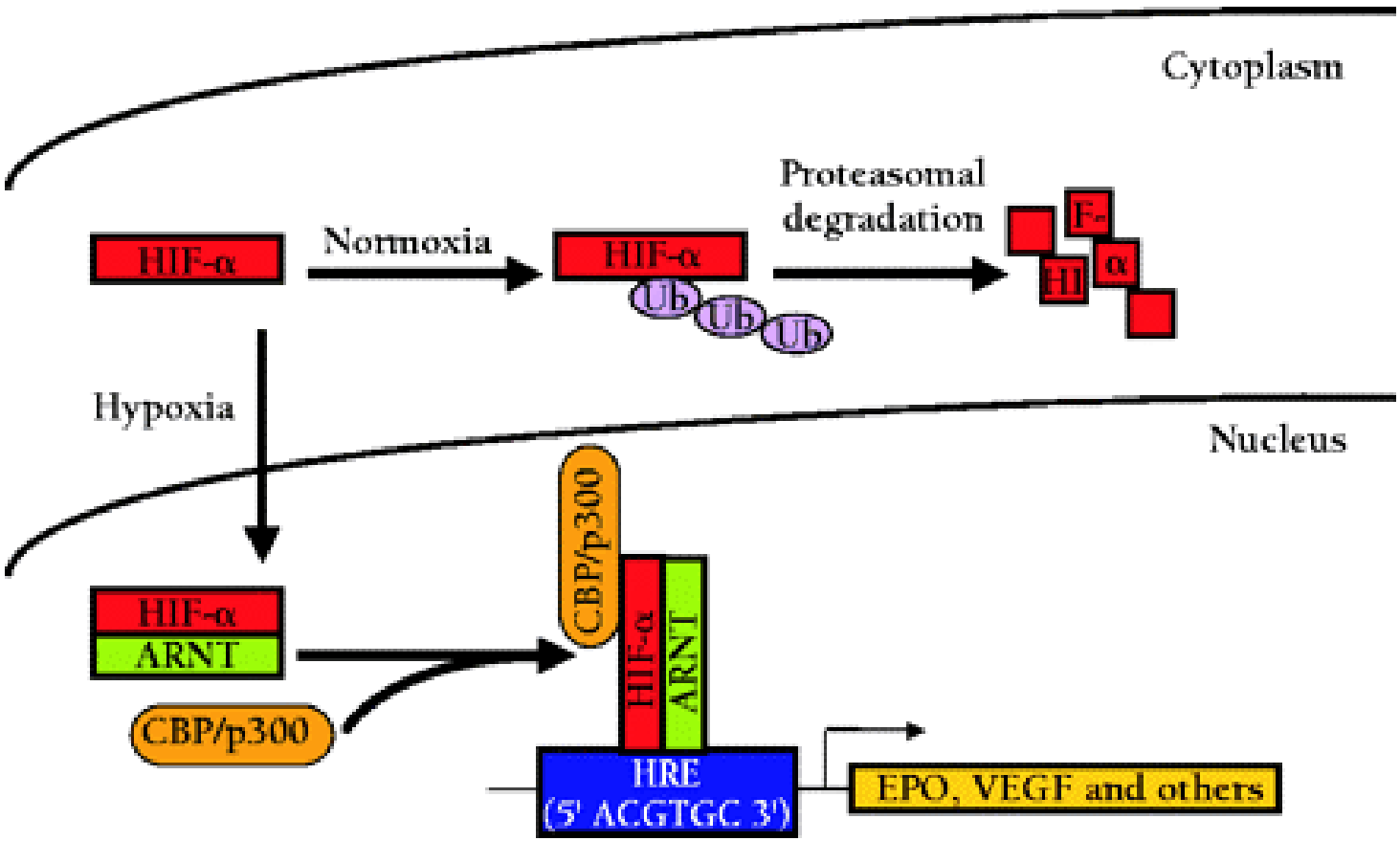
Mahon PC, et al. (2001) Genes Dev. 15:2675–2686.
Lando D, et al. (2002) Genes Dev. 16:1466–1471.



- Under hypoxia, the required O_2 tension for asparaginyl hydroxylase activity is deficient. As a result, CBP/p300 can associate with the unhydroxylated CAD and potentiate transcriptional activtity.

Fedele AO, Whitelaw ML. Peet DJ. (2002) Molecular Interventions. 2:229-243.

If HIF-α is not degraded, it translocates to the nucleus and initiates gene expression



Fedele AO, Whitelaw ML. Peet DJ. (2002) Molecular Interventions. 2:229-243.

Hypoxic conditions stabilization of HIF-1α

- α subunit is induced only when O_2 below ~ 6%
- When there is not enough O_2 present in the cell to hydroxylate HIF-1α by the PHDs, HIF-1α cannot bind to VHL. It then translocates from the cytoplasm to dimerize with HIF-1β subunit in the nucleus (slide 9).
- With the association of the transcriptional co-activators (CBP/P300), the dimer will bind to the hypoxia responsive element (HRE) of HIF target genes and activate the transcription of these genes (slide 9).

Example of genes that are transcriptionally activated by HIF-1

- **More than 60 putative direct HIF-1 target genes have been identified.**

Regulation of HIF-1 activity
p53srj

Glucose metabolism
ENO1, HK1,2, PFKL, GLUT1

Erythropoisis
EPO

Vascular tone
HO1, NOS2,

Iron metabolism
Ceruloplasmin, Transferrin

pH regulation
Carbinic anhydrase 9

Angiogenesis
VEGF, TGF-β3

Apoptosis
NIP3, NIX

Cell survival
ADM, TGF-α

Cell proliferation
IGF-2, p21

Adapted from Semenza GL. (2003) Nature Reviews Cancer (3): 721-732.

HIF-1α can be overexpressed

- HIF-1α is overexpressed in many solid tumors, presumably as a result of hypoxia within the tumor

  Zhong H. et al. (1999) Cancer Res. 59:5830–5835.

  Talks KL. et al. (2000) Am. J. Pathol. 157:411–421

- Genetic alteration in tumor can increase HIF-1 activity by decreased ubiquitination or increase synthesis
  - Loss of function: VHL, P53, PTEN, ARF
  - Gain of function: SRC, ERBB2

  Semenza GL. (2003) Nature Reviews Cancer (3): 721-732.

- It has been suggested that the glycolytic end products lactate and pyruvate may induce HIF-1α accumulation

  Lu H. et al. (2002) J Biol Chem. 277:23111–23115.

- It has been proposed that the observed increase in glycolytic enzyme expression is caused by constitutive HIF-1α activity

  Minchenko A. et al. (2002) J Biol Chem. 277: 6183–6187.

Direct HIF-1 target genes that are particularly relevant to cancer

Metabolic adaptation

ALDA, ENO1, GADPH, GLUT1, GLUT3, GPI, HK1, HK2, LDHA, PFKBF3, PFKL, PGK1, PGM, TPI

Apoptosis resistance

ADM, EPO, ET1, IGF2, NOS2, TGFA

Angiogenesis

EG-VEGF, ENG, LEP, TGF-b3, VEGF, VEGFR2

Invasion/matastasis

AMF, CATHD, CMET, FN1, KRT14, 18, 19, MMP2, UPAR, VIM

Semenza GL. (2003) Nature Reviews Cancer (3): 721-732.

HIF influence diseases

- **The expression of HIF-1α is correlated to tumor vascularity**
- **HIF-1α appears to be involved in fibrinogen-induced VEGF expression in the development of choroidal neovascularization associated with age-related macular degeneration**

  Shiose S. et al. (2004) Graefes Arch Clin Exp Ophthalmol. 242:777-783.

- **Loss of function mutation of the VHL gene leads to HIF-1α accumulation and overexpression of HIF-1α target genes**
- **Von Hippel-Lindau (VHL) disease affected individuals develop a variety of tumors such as Haemangioblastomas of the central nervous system, Clear cell carcinoma of the kidneys, and Pheochromocytoma of the adrenal glands**

  Kaelin Jr WG. (2002) Nat Rev Cancer. 2:673-683.

- **Cardiomyocytes and cardiac stromal cells exhibit a marked potential for a prolonged transcriptional response to ischemia mediated by HIF.**

  Jurgensen JS. et al. (2004) FASEB J. 2004 Sep;18(12):1415-7

Detection of HIF-1α

Protein level

- western blot analysis
- immunohistochemistry
- immunofluorescence

mRNA level

- northern blot analysis

Summary

- HIF-1 is a heterodimeric protein that consists of HIF-1α and HIF-1β
- HIF-1 activates the transcription of more than 60 genes that code for proteins that are involved in angiogenesis, glucose metabolism, cell proliferation/survival and invasion/metastasis
- HIF-1 protein synthesis is regulated by activation of the phosphatidylinositol 3-kinase (PI3K) and ERK mitogen-activated protein kinase (MAPK) pathways or by signalling via receptor tyrosine kinases, non-receptor tyrosine kinases or G-protein-coupled receptors.
- HIF-1 protein degradation is regulated by O_2-dependent prolyl hydroxylation, which targets the protein for ubiquitylation by E3 ubiquitin-protein ligases.
- HIF-1 is overexpressed in human cancers as a result of intratumoral hypoxia as well as genetic alterations.