Review

The role of hypoxia-inducible factors in cancer

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Abstract. Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor that mediates the adaptive responses to hypoxia by effecting the transcription of numerous hypoxia-inducible genes. HIF is frequently overexpressed in solid tumors, and the transactivation of HIF targets in transformed cells provides a distinct survival advantage. Accordingly, the upregulation of HIF correlates with increased progression or aggressiveness of the cancer and poor prognosis. In addition to the induction of HIF by hypoxia, its expression is induced by the loss of tumor suppressors VHL, PTEN, TSC1/2, PML, and SDH, as well as by the increased activity of PI3K and/or MAPK signaling pathways, underscoring the significance of HIF in oncogenesis.

Keywords. HIF, VHL, PI3K, MAPK, PHD.

Expression of HIFs

Hypoxia-inducible factors (HIFs) are heterodimeric transcription factors of the basic helix-loop-helix PAS (Per/ARNT/Sim) family of DNA-binding proteins that induce the transcription of a diverse array of genes to effect the hypoxic response. HIFs are composed of an α subunit and a β subunit of which the most ubiquitously expressed is the aryl hydrocarbon receptor nuclear translocator (ARNT) [1, 2]. HIF activity is regulated at the level of the α subunit, with ARNT being constitutively expressed and stable [3]. There are three HIF-α genes in humans: HIF-1α, HIF-2α, and HIF-3α. There are multiple splice variants of HIF-1α and HIF-3α, with dominant-negative protein products being produced in some tissues [4–9]. The role of the different HIF-α subunits has been investigated in development and in disease processes such as cancer where they are frequently overexpressed. In mice, knockout of HIF-1α or HIF-2α is embryonic lethal, while the mouse knockout of HIF-3α has not yet been addressed. HIF-1α−/− embryos die by day E10.5 of cardiac and vascular malformations [10, 11] and HIF-2α−/− embryos die by day E16.5 of failed cardiac function due to decreased release of catecholamines [12]. In human tissues, HIF-1α messenger RNA (mRNA) expression is generally ubiquitous with high expression in the kidneys, while HIF-2α mRNA is predominantly expressed in the heart, placenta, and lungs [13, 14]. HIF-2α may play a role in development of the tubular system and vascular remodelling as it is also expressed in endothelial cells [15–18]. Human HIF-3α mRNA expression has been found to be highest in the heart, placenta, lung, and skeletal muscle [6].
**HIF expression in cancer**

HIFs are frequently upregulated in cancer and their metastases because transcription of their downstream target genes can promote growth and survival. Increased expression and activity of HIF-α in cancer may occur by loss of tumor suppressors such as VHL, activation of oncogenes, and increased activity of the PI3K and MAPK signalling pathways discussed below. High levels of HIF-1α have been positively correlated with tumor progression and poor prognosis in patients with brain, non-small cell lung carcinoma (NSCLC), breast, oesophageal, stomach, fibrosarcoma, colorectal carcinoma (CRC), prostate, ovarian, uterine, and cervical tumors [19–34]. Overexpression of HIF-2α has been correlated with tumor progression and poor prognosis in patients with NSCLC, head and neck squamous cell carcinoma (HN-SCC), CRC, and VHL–/– clear cell-renal cell carcinoma (CC-RCC) [31, 32, 34–38]. HIF-3α expression is detectable in several human cancer cell lines [6, 39], and while expression of the dominant-negative HIF-3α inhibits endogenous HIF-1-mediated transcription [6, 8], the significance of the full-length HIF-3α for tumor progression is unknown. Generally, HIF overexpression promotes tumorigenesis, but there are several examples to the contrary. Knock-down of HIF-2α in rat glioma tumors reduced apoptosis, and overexpression of HIF-2α reduced growth of these tumors [40], while in VHL–/– CC-RCC xenograft assays, overexpression of HIF-1α inhibited tumor growth and overexpression of HIF-2α promoted tumor progression [37].

**Regulation of HIF-α by PHDs and VHL**

HIF activity is regulated at the level of the α subunit by mechanisms affecting its protein expression, stability, and transcriptional activity. The most well characterized regulated mechanism for HIF-α is its degradation in the presence of oxygen [1]. Oxygen-dependent hydroxylation by a family of prolyl-hydroxylase domain-containing proteins, or PHDs 1–3 [41, 42], at a conserved residue in the oxygen-dependent degradation (ODD) domain mediates binding to the von Hippel Lindau (VHL) tumor suppressor protein, pVHL [6,43–48]. pVHL is the substrate-docking interface for an E3 ubiquitin ligase complex that polyubiquitylates HIF-α, targeting it for degradation by the common 26S proteasome [46–48]. Under conditions of reduced oxygen availability PHDs do not hydroxylate HIF-α [41,42], the VHL E3 ligase does not recognize the α subunit, and it is no longer targeted for ubiquitin-mediated destruction by the 26S proteasome [46–48]. Patients with VHL disease have inherited an inactivating mutation in one allele of VHL and have a subsequent somatic mutation of the remaining allele [49], CC-RCC-causing mutations in VHL patients that prevent VHL from recognizing HIF-α or those that disrupt the formation of a functional E3 ligase complex result in the failure to target HIF-α for efficient oxygen-dependent degradation and a loss of tumor suppressor function for VHL in the kidney [50, 51]. In these VHL patients or individuals with somatic mutations of VHL in the kidney epithelium, patients develop CC-RCC and haemangioblastomas [52–56]. As mentioned, overexpression of HIF-α, particularly HIF-2α, has been implicated in the tumorigenesis of VHL–/– CC-RCC. Interestingly, mutations in tumor suppressors other than VHL may also disregulate HIF-α stability by affecting pVHL’s ability to bind HIF-α. Loss of the succinate dehydrogenase (SDH) or fumarate hydratase (FH) tumor suppressors result in increased levels of succinate and fumarate, respectively, which inhibit PHD activity preventing hydroxylation of HIF-α [57].

**Regulation of HIF-α protein levels by phosphorylation signalling cascades**

Both phosphoinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signalling cascades can regulate HIF-1α protein levels in an oxygen-independent manner. Increased expression of HIF-1α via the PI3K signalling pathway may occur by gain-of-function mutations in upstream positive regulators such as receptor tyrosine kinases and Ras, or loss-of-function mutations in tumor suppressors such as tuberous sclerosis (TSC) 1 or 2 or phosphatase and tensin homolog (PTEN) [58–64]. The PI3K pathway can increase translation of HIF-1α mRNA by both mammalian target of rapamycin (mTOR)-dependent or mTOR-independent mechanisms [58, 61, 65, 66]. As part of a signalling complex, mTOR kinase regulates protein synthesis via S6 kinase-mediated phosphorylation of S6 ribosomal binding protein to increase expression of translational mediators and via activation of eukaryotic initiation factors to increase both cap-dependent and cap-independent translation [66]. The mTOR-dependent mechanism of increased HIF-1α translation is most active under low serum conditions and has been observed in TSC2–/– mouse embryonic fibroblast (MEF) cells and osteosarcoma U2OS cells with TSC2 knock-down [61], in neuroblastoma cells downstream of brain-derived growth factor (BDGF)-mediated activation of the TrkB receptor via PI3K [30], in IGF-2-overexpressing rhabdomyosarcoma cell lines [67], in the MCF7 breast