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INVITED REVIEW

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Hypoxia-responsive transcription factors

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Abstract Hypoxia is a common pathophysiological occurrence with a profound impact on the cellular transcriptome. The consequences of hypoxia-induced or hypoxia-repressed gene expression have important implications in disease processes as diverse as tumour development and chronic inflammation. While the hypoxia-inducible factor (HIF-1) plays a major role in controlling the ubiquitous transcriptional response to hypoxia, it is clear that a number of other transcription factors are also activated either directly or indirectly. In this review, we comprehensively discuss the transcription factors that have been reported to be hypoxia-responsive and the signalling mechanisms leading to their activation. Understanding such events will enhance our understanding of cellular oxygen sensing.

Introduction

Cellular hypoxia occurs when the demand for molecular oxygen necessary to generate ATP levels sufficient to sustain normal physiologic function exceeds the vascular supply. Tissue hypoxia can occur during a diverse array of disease states including, but not limited to, vascular disease, chronic inflammation and cancer. Because molecular oxygen is the primary source of metabolic energy for all eukaryotic cells, it is not surprising that over the course of evolution we have developed the capacity to respond to hypoxic insults with the transcriptional up-regulation of genes that enhance tissue perfusion and anaerobic ATP generation through glycolysis, an event mediated primarily through the hypoxia-inducible factors HIF-1 and HIF-2. Although, primarily, a homeostatic response is directed at the reestablishment of perfusion and tissue oxygenation, this response may be maladaptive and contribute to tumour survival. Gene array analysis has recently revealed significant information regarding global transcriptomic changes in response to hypoxia. These studies have revealed that a significant cohort of alternatively regulated genes that may contribute to hypoxia-induced phenotypic changes are also induced in hypoxia [55, 59]. Together, these studies have demonstrated that hypoxia has a profound effect on the cellular transcriptome, an effect that is likely to be cell-type and cell-state specific. The mechanism(s) by which cells sense hypoxia and transduce this signal to the activation of transcriptional regulators are areas of intense investigation, and current theories include a direct role for oxygen-dependent regulatory enzymes such as prolyl hydroxylases and a role for the generation of reactive oxygen species (ROS). In each case, it is likely that the mitochondria, as the primary site of oxygen consumption, play a major role in the signalling process. In this review, we discuss the various transcription factors that have been demonstrated to be hypoxia-responsive and contribute to the complex transcriptional profile activated by this important physiological and pathophysiological stimulus. It is hoped that taking a global view of hypoxia-sensitive transcription factors may shed light on a general understanding of oxygen-sensing mechanisms.

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Hypoxia-responsive transcription factors

HIF

The hypoxia-inducible factor (HIF) comprises the heterodimeric transcription factors HIFα and HIFβ of

the Per-Arnt-Sim (PAS) family of basic helix-loop-helix proteins [94]. These proteins bind to consensus DNA binding motifs within regulatory promoter regions (hypoxia-responsive elements) of hypoxia-responsive genes. HIF-1α is the most ubiquitously expressed and best characterised of the family and is recognised as a master regulator of hypoxic signalling [82]. HIF-2α is similar in regulation to HIF-1α, but its expression is restricted to certain cell types [24, 92]. HIF-3α is less well characterised but may act as an internal repressor of the HIF system, given that a HIF-3α splice variant encodes IPAS (inhibitor PAS domain protein) [58].

In general, activation of the HIF pathway leads to the induction of an adaptive phenotype. Genes under the control of HIF-1 include those involved in vasodilatation (e.g. the inducible form of nitric oxide synthase iNOS), glycolysis (e.g. glucose transporters GLUT 1 and 3), angiogenesis (e.g. vascular endothelial growth factor VEGF) and enhanced blood oxygenation (e.g. erythropoietin, EPO) [80]. The expression of such genes is of benefit in tissue survival and adaptation in ischaemic disease. However, in cancer, HIF's adaptive role is subverted to a maladaptive state promoting tumour growth, survival and chemotherapeutic resistance [21, 34].

The molecular mechanisms whereby HIF-1 activation occurs in hypoxia have been reviewed extensively elsewhere [80]. Briefly, HIF-1α and HIF-2α are synthesised constitutively but are targeted for degradation in the presence of molecular oxygen by modification of oxygen-dependent degradation domains within the HIF protein. This is carried out by members of the 2-oxoglutarate-dependent dioxygenase superfamily, namely the prolyl hydroxylases (PHD1, PHD2 and PHD3). Hydroxylation of proline residues 402 and 564 of the α-subunit of HIF-1 facilitates interaction with the von Hippel-Lindau tumour suppressor, which targets HIFα for proteasomal degradation. A second oxygen-dependent transactivation domain is regulated in a similar manner by the asparagine hydroxylase known as Factor Inhibiting HIF (FIH) [57]. In hypoxia, oxygen-dependent proline and asparagine hydroxylation are inhibited and HIFα accumulates, translocates to the nucleus and associates with co-activators to regulate transcription. There HIFα binds to the constitutively expressed nuclear protein HIFβ (also known as the aryl hydrocarbon receptor nuclear translocator; ARNT), docks with hypoxia-responsive elements (HREs) in target genes and becomes transactivated.

Recent work has demonstrated an important role for mitochondria in regulating HIF activation. In the steady state (physiological normoxia), the mitochondria consume approximately 90% of available oxygen in the generation of ATP through oxidative phosphorylation in order to meet the metabolic needs of the cell [73]. The residual ~10% is available to the cell for other processes including HIF-1α and HIF-2α degradation. In hypoxia, the mitochondria act like a sink, consuming most available oxygen due to the high affinity of cytochrome c oxidase for molecular oxygen [29]. Thus, there is insufficient oxygen available for HIF-1 hydroxylation by the 2-oxoglutartate-dependent dioxygenase enzymes (which have a relatively high K_m for oxygen) [35]. Inhibition of respiration by nitric oxide (NO) (the endogenous inhibitor of cytochrome c oxidase) in hypoxia can destabilise HIF-1α via redistribution of available oxygen to the cytosol [31]. Thus, HIF-1/2 represent truly oxygen-dependent transcription factors. An alternative model of oxygen sensing involving mitochondrial production of ROS in hypoxia has also been proposed [12]. In this model, it is hypothesised that a paradoxical increase in mitochondrial ROS production at Complex III of the electron transport chain leads to HIF-1 activation [13]. However, the molecular target for ROS which leads to HIF-1 stabilisation has yet to be found.

NFκB

Nuclear factor kappa-B (NFκB) has been studied extensively for its roles in innate immunity, stress responses and cell survival and development and is thought to be a central transcriptional mediator of the inflammatory response [18]. The family of NFκB transcription factors comprise proteins with a highly conserved Rel homology (RH) domain. There are five members of this family: p65, cRel and RelB, which represent the transcriptionally active members and p50 and p52, which are derived from the longer proteins p105 and p100 respectively. The NFκB family of proteins can either homodimerise or heterodimerise to form transcriptional complexes. The most common active dimer complex is that of p50-p65 [18].

NFκB is bound to the repressor molecule IκB in the cytosol in the absence of stimulus. This coupling of proteins masks the nuclear localisation sequence (NLS) of NFκB and sequesters the protein in the cytosolic compartment. Upon stimulation, IκB is targeted for ubiqutination and degradation by specific serine phosphorylation. The NLS of NFκB is then exposed, and NFκB translocates to the nucleus where it carries out its transcriptional activity at specific κB sites within the promoter regions of target genes [18]. A more dynamic role for both NFκB and IκB localisation and shuttling has also been proposed [6]. NFκB-responsive genes include those responsible for encoding inflammatory cytokines, chemokines and cell surface adhesion molecules. A wide variety of stimuli can initiate NFκB, for example, proinflammatory cytokines, bacterial products and ultraviolet light. The convergence point for these disparate stimuli seems to be at the level of the I κ-B kinases (IKKs) which are upstream of IκB phosphorylation [18].

Hypoxia has been demonstrated to activate NFκB in a number of studies [15, 47, 54, 60, 78, 89], however, the signalling mechanism leading to this remains unclear. Target genes for hypoxia-induced NFκB include cyclooxygenase-2 (COX-2), tumour necrosis factor α (TNFα),

interleukin-6 (IL-6) and macrophage inflammatory protein-2 (MIP-2). Tyrosine phosphorylation of IκB [47] by the Ras/Raf kinases downstream from Src has been implicated as a mechanism of NFκB activation in hypoxia [48]. Another model proposes that mitochondria-derived ROS generation in hypoxia may be responsible for NFκB activation [15]. In addition, Zampetaki et al. [105] have demonstrated that hypoxia-induced transactivation is mediated by p42/44 and PI-3-kinase. In summary, while it is clear that NFκB is a hypoxia-responsive transcription factor, multiple specific mechanism(s) of activation may exist.

CREB

The cyclic AMP response element binding protein (CREB) is one of a family of leucine zipper transcription factors regulated by intracellular signalling mechanisms such as cAMP and Ca^{2+}. CREB regulates the expression of a diverse array of genes including those involved in inflammation, metabolism and signal transduction. CREB generally acts as an activator of transcription in a manner mediated through phosphorylation of Ser 133 by protein kinase A (PKA) or calmodulin (CaM) kinase [61]. However, in some cases, CREB may act as a repressor of gene transcription [25]. Recently, it has been demonstrated that CREB-dependent gene expression is altered in response to hypoxia in vitro and in ischaemic disease in vivo.

Acute mild hypoxia in neuronal cells activates CREB through phosphorylation at serine 133 [5]. In an intestinal epithelial cell model, more severe hypoxia results in the phosphorylation-dependent targeting of CREB to ubiquitin-mediated degradation, an event mediated through decreased activity of protein phosphatase 1γ [90]. This event leads to derepression of inflammatory gene expression and thus contribute to inflammatory processes. Interestingly, more prolonged exposure to severe hypoxia results in CREB stabilisation and a resolution of inflammatory gene expression through small ubiqutin-related modifier-1 (SUMO-1) modification [20]. Thus, CREB-dependent gene expression is dependent upon the extent and degree of stimulus.

AP-1

Activating protein-1 (AP-1) is a pleiotropic, dimeric transcription factor involved in diverse cellular functions related to apoptosis, cell proliferation, cell differentiation, catecholamine biosynthesis, inflammation, xenobiotic metabolism, tumour invasion and angiogenesis [83]. AP-1 comprises members of Fos, Jun, ATF (activating transcription factors) and MAF (musculoaponeurotic fibrosarcoma) protein families that can homodimerise or heterodimerise to form the active AP-1 complex that modulates gene expression. The combinatorial complexing of these discrete proteins provides multiple levels of gene expression control. In addition, cell type and differentiation state can dictate the phenotypic outcome, accounting at least in part for how AP-1 can regulate apparently conflicting endpoints such as apoptosis and cell proliferation [62]. AP-1 can be activated by growth factors, pro-inflammatory cytokines, UV radiation and hypoxia. The AP-1 family members have been reviewed extensively [3, 23, 42, 62, 83].

Hypoxia has been shown to activate AP-1 and mediate alterations in gene expression. Genes regulated at least partially by AP-1 in hypoxia include tyrosine hydroxylase [65], VEGF [74], and endothelial NOS (eNOS) [36]. AP-1 co-operates with other transcription factors such as HIF-1, GATA-2, NF-1 and NFκB to complement the activation of hypoxia-sensitive genes [65, 74, 84, 97]. Thus, AP-1 may represent an important facilitator of hypoxia-induced gene expression through interaction with other transcription factors.

The mechanism by which AP-1 is activated in hypoxia has yet to be fully elucidated. However, it appears to be mediated at least in part via a Jun N-terminal kinase (JNK)-dependent pathway [66]. The oxygen-sensing mechanism upstream of JNK remains unclear. AP-1 is a redox-sensitive transcription factor and it has been suggested that hypoxia alters cellular metabolism and consequently the redox environment in the cell, thus favouring AP-1-mediated transcriptional activation [36]. This is likely to be a complex process, given AP-1's apparent activation by oxidants [36] and anti-oxidants alike [63]. Another signalling mechanism proposed is hypoxia-induced modulation of intracellular Ca^{2+} levels upstream of AP-1 activation [27, 65, 71, 74]. This increase is thought to activate AP-1 independently of HIF. Other reports demonstrate a role for non-receptor tyrosine kinases in propagating the hypoxic signal from G protein-coupled receptors based on results implicating a role for Src (non-receptor tyrosine kinase) and Ras [71].

p53

p53 is a tetrameric transcription factor involved in cell-cycle arrest and apoptosis. It is responsible for the induction of a number of pro-apoptotic genes including Bax, Bid, PUMA and Apaf-1 (apoptotic protease activating factor-1) [86]. It has a number of triggers including UV light, X-irradiation, DNA damage, low extracellular pH, hypoxia and heat shock [1]. p53 is the most frequently mutated gene in cancer, with over 50% of tumours exhibiting a mutation. p53 has a short half-life and is usually targeted for degradation by MDM2, a ubiquitin ligase that competes for p53's DNA binding sequence and co-activators [104].

HIF-1α can bind to MDM2 both in vitro and in vivo resulting in p53 stabilisation [2, 17]. A direct interaction between p53 and HIF-1-derived fragments has also been reported [33], although this interaction is controversial

[32, 95]. In addition, p53 has been shown to promote MDM2-mediated ubiquitination and subsequent proteasomal degradation of HIF-1α [72].Further interactions can occur at the level of competition for the shared co-activator p300 [72, 79]. The nature of the HIF-1α-p53 interaction is dependent on the phosphorylation status of HIF-1α, with dephosphorylated HIF-1α deviating from classical HIF-1 signalling and binding to p53 [87]. MDM2 expression is increased in hypoxia in a HIF-1-independent manner [107], although it has also been demonstrated to be decreased in hypoxia [2]. Thus, the complex interactions between HIF-1α and p53 are important considerations particularly in the hypoxic environment of the tumour. Hypoxia can contribute to the metastatic potential of tumours by modulating MDM2 and p53, whilst mutated p53 can contribute to the angiogenic switch by the amplification of normal HIF-1α responses.

In an interesting caveat, recent studies have indicated that neither hypoxia nor anoxia alone are sufficient to drive p53 accumulation and that glucose deprivation and acidosis secondary to hypoxia are essential co-incidental events [70]. Furthermore, ROS have also been reported to be involved in p53 regulation in hypoxia [16].

SP-1 and SP-3

SP1 and SP3 are ubiquitous transcription factors of the Sp/XKLF transcription factor family that are involved in basal transcription and housekeeping gene expression [75]. They have identical sequence binding motifs, but can display differential activity including parallel or divergent activity, depending on the promoter. Levels of SP1 are regulated to an extent by mRNA expression, but further regulation can be imposed by proteasomal degradation, for example, in response to nutrient starvation and adenylate cyclase stimulation [8].

Several classically hypoxia-responsive genes such as EPO and VEGF have SP1/SP3 binding sites within promoter regions that are thought to facilitate transcriptional activation [51, 77]. COX-2 is also hypoxia-responsive. In an experiment investigating SP1 and SP3 involvement in COX-2 expression, hypoxia increased nuclear localisation of SP1 but didn't change SP3 levels [96]. In a separate study, decreased binding of SP1 to the UDP-glucose dehydrogenase (UGDH) promoter occurred following hypoxic exposure [7]. A further study has reported progressively decreased SP3 expression and DNA binding to a glycolytic gene promoter in hypoxia. SP1 levels remained unchanged in this study [22]. In addition, several studies have hypothesised the involvement of SP1 in facilitating promoter activation in hypoxia [51, 53, 64]. Kaluz et al. [44] have reported a novel hypoxia-responsive enhancer for carbonic anhydrase IX. This gene is activated differentially in mild and severe hypoxia, SP1/SP3 being absolutely required in mild hypoxia, while SP1/SP3 significantly up-regulates the predominantly HIF-1-mediated hypoxic induction in severe hypoxia. SP1 also interacts with Smad3 when the TGFβ pathway is active to facilitate full hypoxic inducibility of EPO [77].

While the signalling mechanisms leading to hypoxia-induced SP1/SP3 activity remain unknown, SP1 has been reported to be redox-sensitive [50]. However, no role for this signalling pathway has been reported for SP1/SP3 in hypoxia.

Egr-1

Early growth response-1 (Egr-1) is a zinc finger transcription factor involved in a number of early responses to a variety of stimuli including growth factors, hormones, neurotransmitters and hypoxia. Egr-1 binds with high affinity to a consensus DNA element to modulate the expression of genes involved in synaptic plasticity, cell growth and survival, extracellular matrix remodelling and thrombosis [91]. The latter functions are of particular interest in relation to hypoxia-induced Egr-1 expression. Egr-1 nuclear localisation is enhanced under the conditions of hypoxia. This binding has been found to be necessary for hypoxic induction of the pro-coagulant tissue factor using experiments involving Egr-1 null mice. In addition, Egr-1 activation in hypoxia is independent of HIF [103]. Further studies have confirmed that the increase in Egr-1 reflects de novo biosynthesis [100] and several groups have reported an increase in Egr-1 message and/or protein under conditions of hypoxia [4, 41, 68, 100]. Up-regulation of Egr-1 is dependent upon the degree and severity of hypoxia [98]. Dissection of the molecular events upstream of Egr-1 activation has led to the identification of members of the protein kinase C (PKC) family as crucial triggers in hypoxia-induced Egr-1 activity and subsequent gene expression. PKCβ null mice reveal markedly decreased Egr-1 levels in response to hypoxia [99]. Furthermore, PKCα is implicated in Egr-1 gene induction in endothelial cells in hypoxia. Ras/Raf/ERK1/2 are active downstream from PKCα in this model [56]. Hypoxia-induced Egr-1 is an important regulatory event in contributing to the pathogenesis of pulmonary thrombosis and vascular remodelling [81, 101].

NF-IL6/ C/EBPβ

NF-IL6 (nuclear factor for interleukin 6) is a member of the C/EBP (CCAAT/enhancer-binding protein) family of transcription factors and is also known as C/EBPβ.Its expression can be regulated transcriptionally and post-transcriptionally, with the *Cepb* gene being translated into multiple protein isoforms [30]. It is capable of dimerisation with several transcription factors of different origin including CREB, Fos and Jun. NF-IL6 harbours a negative regulatory domain that hampers full activation. Phosphorlyation of NF-IL6 releases this constraint and allows transactivation. This phosphorylation can be

mediated by a number of different kinases including: PKA, CaM kinase (CaMK), mitogen-activated protein kinase (MAPK) and PKC. NF-IL6 has a number of important physiological roles in mammary gland development and regulation of the anti-inflammatory cytokine IL-6. Stimulation of NF-IL6 has been demonstrated in response to inflammatory agents such as phorbol myristate acetate (PMA), lipopolysaccharide (LPS), IL-6 and interferon-γ (IFNγ), in addition to hormones and hypoxia [88].

In an hypoxic inflammatory environment, NF-IL6 plays a central role in IL-6 production, which may in turn contribute to the attenuation of the pro-inflammatory phenotype [81]. mRNA levels of IL-6 increase in endothelial cells in response to hypoxia. Supershift analysis of the nuclear binding has revealed enhanced binding by NF-IL6 [102] and similar trends are seen in cardiac myocytes exposed to hypoxia, where NF-IL6 co-operates with NFκB in the production of IL-6. There is a definite degree of cell specificity for these responses as fibroblasts fail to yield the same responses [60]. Thus, there is strong evidence for NF-IL6's role in IL-6 regulation in hypoxia. This is most likely triggered through one of the kinase cascades upstream from NF-IL6 activation.

Other transcription factors

Limited work has been published on other transcription factors reportedly involved in transcriptomic responses to hypoxia. For completeness, these are outlined below.

Related transcriptional enhancer factor-1 (RTEF-1) is a member of the TEF-1 family that can regulate gene expression particularly in cardiac and skeletal muscle cells. RTEF-1 is up-regulated in hypoxic endothelial cells and may modulate VEGF transcription through binding to an SP-1 site in the promoter region [85].

GATA-2 has been associated with negative regulation of cytokine-induced EPO gene transcription [49]. This finding is supported by the evidence that EPO mRNA is stimulated in the presence of an antisense oligonucleotide for the GATA element regardless of the EPO stimulus [37]. The first evidence for the role of GATA factors in hypoxia was the demonstration that GATA-2 interacts physically with other transcription factors (HIF-1, AP-1 and p300/CBP) and is necessary for full expression of the endothelin-1 gene. However, hypoxia does not affect the relative abundance or binding activity of GATA-2 [97]. Mouse GATA-2 also inhibits a hypoxia-induced EPO-luciferase reporter construct in the mouse [38].

The signal transducers and activators of transcription (STAT) family of transcription factors are activated by phosphorylation on tyrosine residues in response to a variety of stimuli including cytokines and hypoxia. STAT5 is phosphorylated under the conditions of hypoxia resulting in increased binding to the β-casein gene promoter [43].

Mammalian achaete-scute homologous protein-2 (Mash-2) is involved in placental trophoblast development. Elevated cytoplasmic levels of Mash-2 have been observed in hypoxic trophoblasts and are decreased in normoxia [40]. Another study reports that hypoxia-induced inhibition of aromatase expression is governed by Mash-2 [39].

Growth arrest and DNA damage-153 (GADD153/CHOP-10) is a pro-apoptotic transcription factor that can be activated by hypoxia. GADD153 mRNA levels increase in response to hypoxia independently of HIF-1 [11]. Calcium channel blockade, G protein inhibition and PKC down-regulation abrogate hypoxia-induced GADD153 expression without affecting basal GADD153 expression in pulmonary artery smooth muscle cells while antioxidants have no effect [19]. However, in adipocytes, hypoxia-induced mitochondrial ROS are thought to be the signal for increased GADD153 expression and antioxidants partially prevent hypoxic induction of GADD153 in this study [11]. Interestingly, a recent communication has reported anoxia-specific induction of GADD153 in human cancer cells, which is not seen in hypoxia. Clearly the oxygen-sensing mechanism leading to GADD153 activation has yet to be fully described.

A number of transcription factors are activated downstream of HIF-1. DEC1 (differentially expressed in chondrocytes 1) [STRA13 (stimulated with retinoic acid 13), SHARP2 (enhancer-of-split and hairy-related protein 2)] and DEC2 are hypoxia-inducible transcription factors that are HIF-1-dependent [67]. ETS1 is a hypoxia-inducible transcription factor that has been shown to be HIF-1-dependent. ETS1 plays a role in cancer invasion and angiogenesis [69].

The Smad signalling pathway has recently been shown to interact with HIF-1 for the induction of hypoxia-responsive genes such as VEGF, EPO and transforming growth factor-β2 (TGFβ 2) [76, 77, 106]. Physical interaction between Smad3 and HIF has been confirmed by co-immunoprecipitation studies [76]. Exposure of human vascular endothelial cells (HUVECs) to hypoxia results in phosphorylation and nuclear transportation of Smad2 and Smad3 proteins as well as in stimulation of transcriptional activities of Smad3 and HIF-1α [106]. In addition, under conditions of hypoxia, co-operative binding of Smad3/4 with HIF-1 occurs in the EPO gene. This synergetic interaction only occurs, however, when the TGFβ signalling pathway is mediating cross-talk between SP1 and HIF-1 via Smad3 [77]. Thus, Smad proteins can modulate hypoxic responses actively when TGFβ signalling is active.

Discussion

In summary, hypoxia activates a diverse array of transcription factors and thus has a profound impact on the cellular transcriptome (Figure 1). A number of

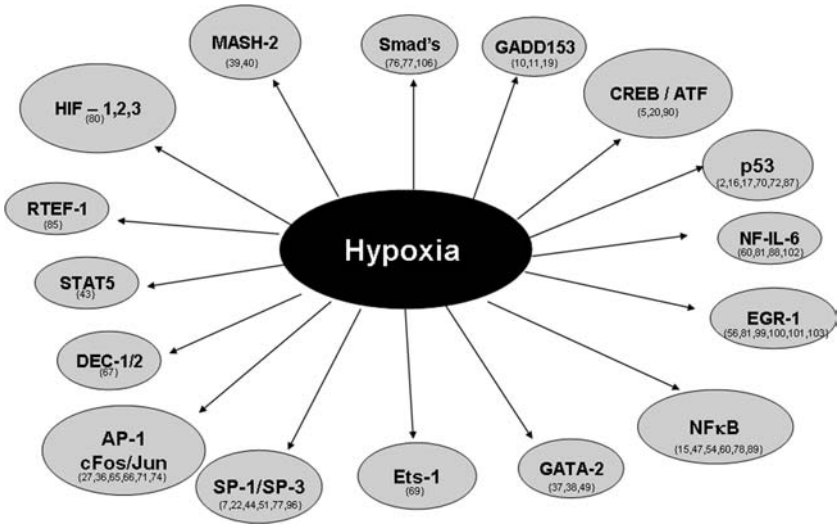

Fig. 1 Diagram representing hypoxia responsive transcription factors. Included below each transcription factor, in brackets, are some of the major relevant references

complexities exist which determine the PO_2 at which a cell perceives hypoxia, primarily the cellular oxygen demand as determined by metabolic activity and the cellular levels of the endogenous inhibitor of respiration, NO. Because of these issues, it is likely that the degree and nature of the global transcriptomic response to hypoxia in vivo is both cell-type and cell-state specific. In general, however, the HIF pathway is ubiquitous and represents a truly oxygen-dependent transcription factor as a result of the absolute dependency of proline/asparagine hydroxylation modification on molecular oxygen availability.

The mechanism of hypoxia-sensing that signals to other hypoxia-responsive transcription factors remains less clear. A number of such transcription factors are responsive to alterations in cellular redox potential. Furthermore, ROS have been implicated in their activation under conditions of hypoxia [12–15, 46]. However, significant controversy still exists as to whether significant increases in ROS occur in hypoxia, with a number of studies reporting a positive correlation [12–15, 46] while others report a negative correlation [9, 26, 28, 45, 93]. Hypoxia can also alter classical signalling pathways such as intracellular cAMP and calcium levels. In addition, signalling kinases outlined above and reported to be activated in hypoxia include PKA, PKC, CaMK, JNK, Src, p38 and p42/44. Thus, while HIF is a major factor in determining the cellular response to hypoxia, a significant number of secondary pathways may modulate the global transcriptomic response. Furthermore, given the diverse array of transcription factors activated, it is likely that a number of signalling pathways may be induced as a cell undergoes the transition from normoxia to hypoxia. It is also likely that the degree and extent of exposure to hypoxia along with cellular oxygen demand will dictate the primary signalling pathways activated in a given hypoxic circumstance. Thus, the transcriptional outcome of hypoxia probably depends on the degree of hypoxia experienced and the cellular requirement for oxygen. Further studies are required to determine the series of signalling events as a cell undergoes the transition from normoxia to mild, moderate, severe and lethal hypoxia.

In conclusion, it is becoming clear that the mitochondria, as the primary site of oxygen consumption and ROS production, play a pivotal role in oxygen sensing. ATP depletion, not outlined in this review, which also occurs during hypoxia, can activate the AMP kinase pathway, leading to alterations in transcription [52]. In all likelihood, the signalling pathways outlined in this review will interact to mediate the global cellular transcriptomic response to hypoxia.

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