REVIEW

Regulation of hypoxia-inducible factor 1 and the loss of the cellular response to hypoxia in diabetes

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Abstract Diabetes is frequently associated with hypoxia and is known to impair ischaemia-induced neovascularisation and other forms of adaptive cell and tissue responses to low oxygen levels. Hyperglycaemia appears to be the driving force of such deregulation. Recent data indicate that destabilisation of hypoxia-inducible factor 1 (HIF-1) is most likely the event that transduces hyperglycaemia into the loss of the cellular response to hypoxia in most diabetic complications. HIF-1 is a critical transcription factor involved in oxygen homeostasis that regulates a variety of adaptive responses to hypoxia, including angiogenesis, metabolic reprogramming and survival. Thus, destabilisation of HIF-1 is likely to have a negative impact on cell and tissue adaptation to low oxygen. Indeed, destabilisation of HIF-1 by high glucose levels has serious consequences in various organs and tissues, including myocardial collateralisation, wound healing, renal, neural and retinal function, as a result of poor cell and tissue responses to low oxygen. This review aims to integrate and summarise some of the most recent developments, including new proposed molecular models, on this research topic, particularly in terms of their implications for potential therapeutic approaches for the prevention or treatment of some of the diabetic complications characterised by impaired cellular and tissue responses to hypoxia.

Abbreviations

CBP	CREB Binding Protein
CHIP	Carboxy terminus of HSP70-interacting protein
CXCR4	C-X-C chemokine receptor type 4
DMOG	Dimethyloxalylglycine
DFO	Desferrioxamine
eNOS	Endothelial NOS
EPC	Endothelial progenitor cell
FIH-1	Factor-inhibiting HIF-1
GLO1	Glyoxalase 1
HIF-1	Hypoxia-inducible factor 1
HSP	Heat-shock protein
MAPK	Mitogen-activated protein kinase
NOS	Nitric oxide synthase
PHD	Prolyl hydroxylase
PI3K	Phosphoinositol 3-kinase
SDF-1	Stromal cell-derived factor-1
SUMO	Small ubiquitin-like modifier
TCA	Tricarboxylic acid cycle
VHL	von Hippel–Lindau protein
VEGF	Vascular endothelial growth factor

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Regulation of hypoxia inducible factor-1

At low oxygen levels, cells activate a number of critical pathways to cope and survive under hypoxic conditions. Most of these pathways are regulated by the transcription factor hypoxia-inducible factor 1 (HIF-1), and this regula-

tion appears to largely rely on the ability of cells to use oxygen deprivation as a signal to control HIF-1 stability and transcriptional activity. Active HIF-1 is a heterodimer composed of a constitutively produced HIF-1β subunit, which is stable irrespective of the oxygen level, and a labile HIF-1α subunit [1]. Canonically, it is assumed that regulation of HIF-1α is indeed the critical event implicated in the HIF-mediated cellular response to low oxygen, as HIF-1α is highly induced by hypoxia [2, 3]. The HIF-1α subunit is virtually undetectable under normoxic conditions, since it is rapidly degraded by the ubiquitin–proteasome pathway [4, 5]. Under normoxic conditions, HIF-1α has a very short half-life of less than 5 min, being continuously synthesised and degraded [6, 7]. It is well established that under normal oxygen levels, HIF-1α is hydroxylated on proline residues 402 and 564 in the oxygen-dependent degradation domain by specific prolyl hydroxylases (PHDs) [8–12] that require oxygen and 2-oxoglutarate, as co-substrates, and iron (Fe^{2+}) and ascorbate, as co-factors [13–15]. The use of iron by these enzymes explains the hypoxia-mimetic effects of iron antagonists and chelators, such as desferrioxamine (DFO) and cobalt chloride [14, 15]. Although 2-oxoglutarate, a tricarboxylic acid (TCA) cycle intermediate, is essential for the activity of PHDs because of its role in the coordination of iron in the catalytic core, other TCA cycle intermediates such as succinate and fumarate appear to inhibit PHDs by competing with 2-oxoglutarate for binding to the active site [16, 17]. Once hydroxylated, HIF-1α is recognised by the von Hippel–Lindau protein (VHL), which is part of an ubiquitin ligase complex known as E3 ligase complex that targets HIF-1α for polyubiquitination and subsequent proteasomal degradation [18–21]. In addition to VHL, the E3 ligase complex is formed by the RING-finger protein RBX1, which is thought to recognise a cognate E2, as well as several adaptor proteins, such as elongin B, elongin C and cullin 2 [22, 23] (Fig. 1). The asparagine 803 residue of HIF-1α is also hydroxylated under normoxic conditions by a specific asparagine hydroxylase named factor-inhibiting HIF-1 (FIH-1), which impairs the interaction of the transcriptional co-activators p300/CREB binding protein (CBP) with the HIF-1α C-terminal transactivation domain [24–26] (Fig. 1). This leads to further repression of the transcriptional activity of HIF-1. Like PHDs, FIH-1 requires 2-oxoglutarate, iron, ascorbate and dioxygen to induce hydroxylation; however, as opposed to PHDs, FIH-1 is not inhibited by intermediates of the TCA cycle [16, 27, 28]. When oxygen becomes limited, the proline residues are no longer hydroxylated and HIF-1α escapes degradation, accumulating in the cell. Subsequently, HIF-1α is translocated into the nucleus, where it dimerises with HIF-1β and binds to a core pentanucleotide sequence (5′-RCGTG-3′) in hypoxia-responsive elements of the promoter or enhancer

sequences of target genes [3, 29]. Ultimately, HIF-1 activates the expression of numerous genes that help cells to survive at low oxygen levels. In addition, p300/CBP interact with HIF-1α, due to inhibition of Asn803 hydroxylation, increasing the transcriptional activity of HIF-1 (Fig. 1).

Changes in gene expression directly or indirectly regulated by HIF-1 extend to more than 100 genes, which are involved in a plethora of adaptation and survival mechanisms, such as angiogenesis, anaerobic glucose metabolism, erythropoiesis, cell growth, differentiation, survival and apoptosis. One of the best-known target genes induced by HIF-1 is *VEGF*, which encodes vascular endothelial growth factor [29–31], a potent endothelial cell mitogen that is crucial for the angiogenic process. In addition, HIF-1 also induces the expression of the genes for nitric oxide synthase (NOS), haem oxygenase and endothelin [32–34], which play a role in the maintenance of vascular tone and integrity. For example, increased production and activation of endothelial NOS (eNOS) results in increased levels of NO, which triggers mobilisation of bone marrow endothelial progenitor cells (EPCs) into the circulation [35, 36]. Other genes induced by HIF-1 include those for multiple enzymes responsible for shifting the metabolism toward anaerobic glycolysis, such as phospho-glycerate kinase-1, lactate dehydrogenase A and pyruvate dehydrogenase kinase, since oxidative phosphorylation is compromised by reduced oxygen availability. Moreover, HIF-1 also upregulates a more efficient isoform of cytochrome oxidase and increases the expression of *GLUT1* (also known as *SLC2A1*) and *GLUT3* (also known as *SLC2A3*). Interestingly, the glucose metabolite pyruvate appears to regulate the levels of HIF-1α in cancer cell lines by inhibiting PHD-mediated hydroxylation of HIF-1α, contributing to a positive feedback control mechanism (reviewed in [37]). Other crucial proteins upregulated by HIF-1 are C-X-C chemokine receptor type 4 (CXCR4) and the CXCR4-ligand stromal cell-derived factor-1 (SDF-1) that control adhesion, migration and homing of EPCs, required for the formation of new blood vessels [38, 39] (Fig. 1).

Although the canonical pathway for HIF-1α regulation is reliant upon the PHD- and VHL-dependent degradation of the protein, there is evidence to suggest that the degradation of HIF-1α may also occur through alternative pathways. For example, receptor of activated protein kinase C 1 (RACK1) binds to HIF-1α and elongin C, competing with heat-shock protein (HSP) 90 which stabilises HIF-1α, thereby promoting HIF-1α ubiquitination and degradation in an oxygen-independent manner [40]. Additionally, histone deacetylase inhibitors also appear to induce VHL-independent proteasomal degradation of HIF-1α via a mechanism that involves hyperacetylation of HSP90, disruption of the interaction of HSP90 with HIF-1α and formation of immature HIF-1α–
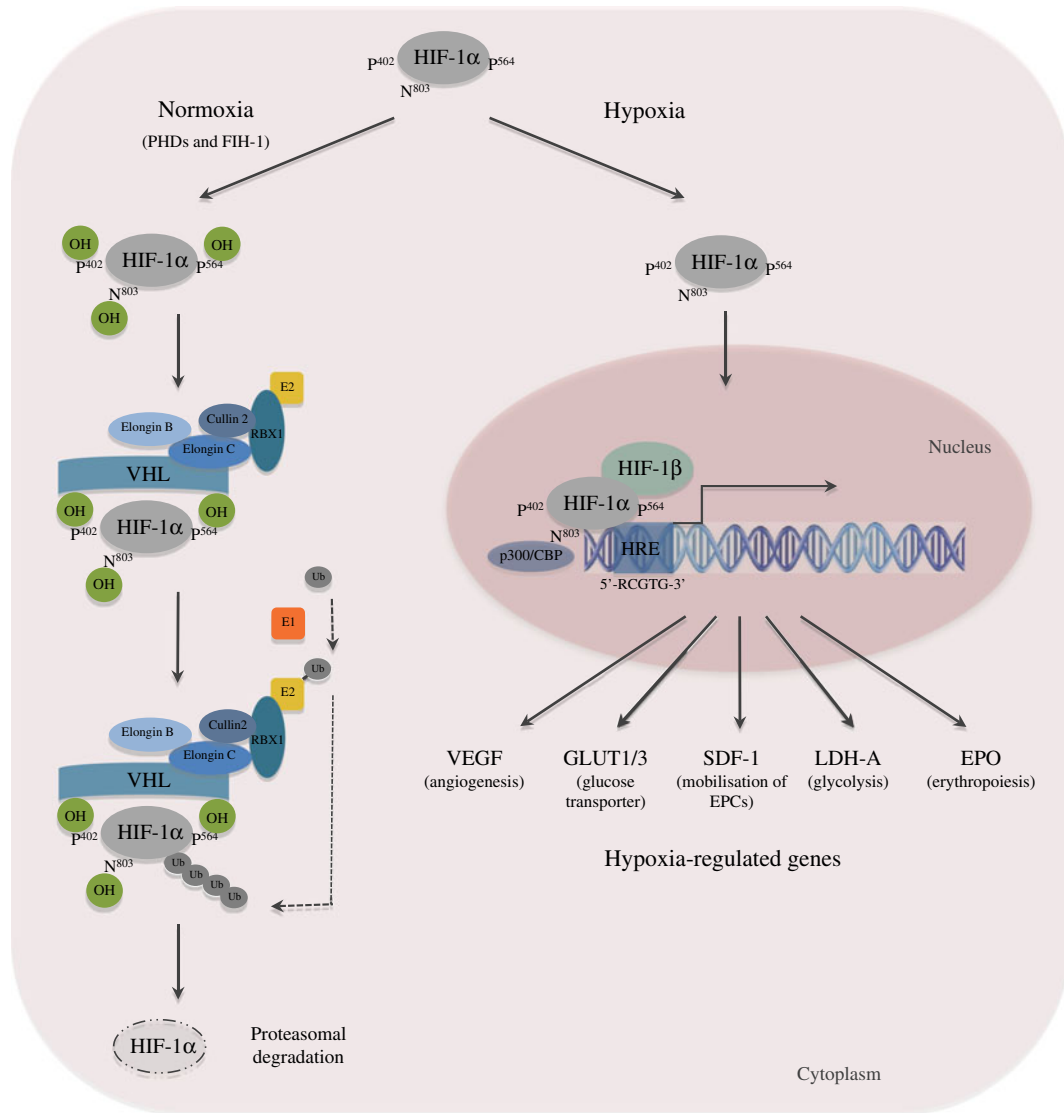
Fig. 1 Schematic representation of the canonical pathway of HIF-1 regulation. Under normoxic conditions, the proline residues 402 (P^{402}) and 564 (P^{564}) of HIF-1α are hydroxylated by specific PHDs. This hydroxylation is recognised by VHL, which is part of an E3 ligase complex composed of elongin B, elongin C, cullin 2 and Rbx1. Together with E1 (ubiquitin-activating) and a specific E2 (ubiquitin-conjugating) enzymes, this ubiquitin–ligase complex induces HIF-1α polyubiquitination (denoted by Ub) and its subsequent degradation by the proteasome. In addition, asparagine 803 (N^{803}) of HIF-1α is hydroxylated by FIH-1, which inhibits the interaction of HIF-1α with the co-activators p300/CBP, reducing the transcriptional activity of HIF-1. When oxygen becomes limited, HIF-1α is no longer hydroxylated, preventing its degradation. Thus, HIF-1α accumulates in the cell and is translocated into the nucleus, where it dimerises with HIF-1β and interacts with p300/CBP. Subsequently, HIF-1 binds to the hypoxia-responsive elements (HREs) of the promoter or enhancer sequence of the target genes, leading to its transcriptional activation. The target genes include those that encode the proteins VEGF, erythropoietin (EPO), SDF-1, GLUT1/3 and lactate dehydrogenase-A (LDH-A), which help cells to cope with the stress induced by low oxygen levels

HSP70 complexes [41]. More recently, the ubiquitin ligase known as carboxy terminus of HSP70-interacting protein (CHIP) was also implicated in HIF-1α ubiquitination and degradation by the proteasome during prolonged hypoxia [42] and in conditions of increased availability of methylglyoxal [43], through a mechanism dependent on molecular chaperones. Methylglyoxal is a highly reactive α-oxoaldehyde, formed mainly as a by-product of glycolysis [44], which suggests that its availability is increased under conditions of high glucose or prolonged hypoxia. Some reports have further suggested that p53 may also target HIF-1α for Mdm2-mediated ubiquitination and proteasomal degradation [45, 46]. SMAD-specific E3 ubiquitin protein ligase 2 (SMURF2) [47] and hypoxia-associated factor [48] have also been suggested to regulate oxygen- and VHL-independent HIF-1α degradation mechanisms.

HIF-1α has also been shown to be regulated by deubiquitination mechanisms by the VHL-interacting deubiquitinating enzyme 2 (VDU2 or Usp20). In contrast to VDU1 (Usp33), VDU2 can specifically deubiquitinate and stabilise HIF-1α and induce expression of HIF-1 target genes [49].

SUMOylation, a post-translational modification that corresponds to covalent conjugation of SUMO (small ubiquitin-like modifier) to target proteins in a manner similar to ubiquitination, has also been implicated in the regulation of HIF-1α and the response to hypoxia, as well as in many other cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability and stress responses. Findings suggest that under hypoxic conditions, HIF-1α might be deSUMOylated by the SUMO-specific isopeptidase SENP1, preventing HIF-1α degradation and leading to activation of its target genes [50]. However, the role of SUMO in HIF-1α regulation is still controversial, as it has also been shown that modification of HIF-1α by SUMO may be involved in its stabilisation. Indeed, RWD-containing SUMOylation Enhancer (RSUME) has been shown to induce SUMO conjugation and stabilisation of HIF-1α [51]. It has also been reported that hypoxia-induced SUMOylation of HIF-1α reduces its transcriptional activity without affecting its half-life [52]. Thus, the precise function of SUMO in the regulation of HIF-1α remains to be elucidated.

Phosphorylation signalling cascades regulate HIF-1α in an oxygen-independent manner. Both phosphoinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways can regulate HIF-1α protein levels and transactivation. For example, increased expression of *HIF-1α* via PI3K pathway may occur by gain-of-function mutations in upstream positive regulators, such as receptor tyrosine kinases (RTKs) and Ras, or loss-of-function mutations in tumour suppressors, such as phosphatase and tensin homologue (PTEN) (reviewed in [53]). Of significance, MAPK-induced phosphorylation of HIF-1α further recruits p300/CBP to HIF-1α, increasing HIF-1 activity [54]. Moreover, phosphorylation of HIF-1α also appears to protect the protein from exportin-dependent nuclear export [55].

These and other findings clearly indicate that control of HIF-1α is very complex and many important questions regarding its regulation remain unanswered.

Loss of the cellular response to hypoxia in diabetes

Both hyperglycaemia and hypoxia are important hallmarks of diabetic complications and appear to elicit several deleterious effects, leading to complications such as diabetic retinopathy, poor wound healing, neuropathies, cardiovascular and renal diseases. A feature that character-ises many of these complications is endothelial dysfunction, mainly resulting from impaired ischemia-driven neovascu-larisation. It has consistently been observed that diabetic animals have decreased vascular density following hind limb ischaemia [56–58] and impaired wound healing [59, 60]. Indeed, it has been extensively shown that ischaemia-induced production of eNOS, SDF-1, CXCR4, VEGF and other growth factors is decreased in diabetic tissues and in hyperglycaemia [38, 60, 61]. This is likely to contribute to decreased function, mobilisation and recruitment of EPCs and other circulating angiogenic cells to injured areas, as well as to poor growth, proliferation and adhesion of endothelial cells [57, 61–66] (Fig. 2). Of significance, these findings were mostly derived from analysis of samples from type 1 and type 2 diabetic patients [63, 64, 66], as well as from studies using *in vitro* culture systems [61, 67] and diabetic animal models, such as *db/db* [57, 59, 63, 68] and streptozotocin-treated animals [61, 62].

In coronary heart disease, mRNA and protein levels of VEGF and its receptors VEGFR1 and VEGFR2 in the myocardium were found to be decreased by 40–70% both in diabetic rats and in insulin-resistant non-diabetic rats. Moreover, a twofold reduction in VEGF and VEGFR2 was observed in ventricles from diabetic patients compared with levels in ventricles from non-diabetic donors [69]. In addition, decreased levels of VEGF in the renal glomeruli were correlated with podocyte cell death, diminished tissue repair and progression of renal disease in diabetic patients [70]. In animal models, low levels of VEGF have also been associated with diabetic peripheral neuropathy [71] (Fig. 2).

Importantly, induction and release of growth factors was found to reduce the clinical symptoms and tissue damage that occur in many ischaemia-associated diabetic compli-cations, such as in coronary artery and peripheral limb diseases, as well as to improve wound healing in diabetic patients (reviewed in [72]). Indeed, local VEGF supply was shown to decrease apoptosis, as well as to improve EPC function, migration and capillary density in diabetic cardiomyopathy [73]. In addition, VEGF-based gene therapy protects nerve conduction [74], improves sensory perception [75], preserves autonomic function and reduces nerve fibre loss [76]. Several gene therapy approaches known to indirectly increase VEGF production improve hypoxia-induced wound healing and angiogenic responses in diabetic animals [57, 63, 77].

A number of independent reports have suggested that cellular adaptation to low oxygen is compromised in the presence of hyperglycaemia, culminating in increased cell death and tissue dysfunction. For example, blood glucose levels showed a linear relationship with fatal outcome in response to an acute hypoxic challenge (i.e. acute myocardial infarction) [67, 78]. It was further shown that AGEs, formed from dicarbonyls such as methylglyoxal, attenuate the
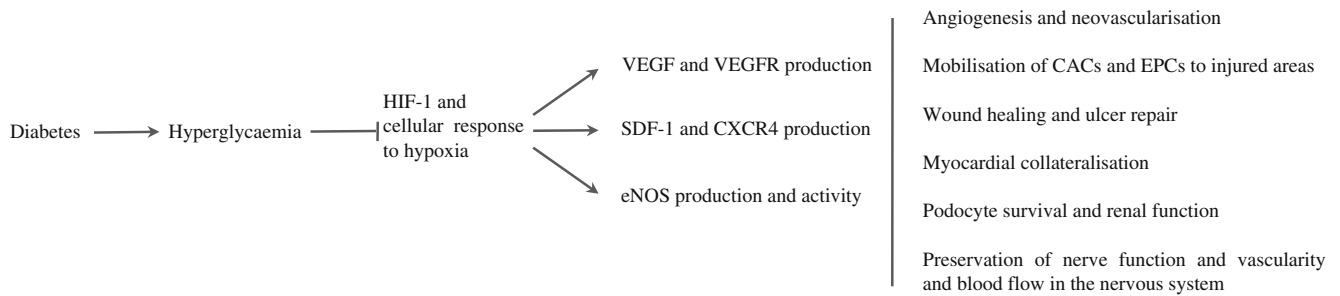
Diabetes ⟶ Hyperglycaemia ⟶| HIF-1 and cellular response to hypoxia ⟶ VEGF and VEGFR production

SDF-1 and CXCR4 production

eNOS production and activity

Angiogenesis and neovascularisation

Mobilisation of CACs and EPCs to injured areas

Wound healing and ulcer repair

Myocardial collateralisation

Podocyte survival and renal function

Preservation of nerve function and vascularity and blood flow in the nervous system

Fig. 2 Impairment of hypoxia-induced responses in diabetic conditions. The cellular response to hypoxia has been shown to be impaired in diabetic conditions and hyperglycaemia appears to be the critical event implicated in such deregulation, most likely as a result of destabilisation of HIF-1. Impairment of the regulation of HIF-1 has several deleterious consequences, including decreased production of VEGF and VEGFR, SDF-1 and CXCR4 and decreased production angiogenic response *in vitro* [79], while in diabetic mice, and activity of eNOS. All of these proteins are required for an appropriate response to hypoxia, these being the driving forces of neovascularisation, myocardial collateralisation, wound healing, renal and neural function and EPC mobilisation to injured areas. Thus, a decrease in their levels has detrimental consequences for cell and tissue adaptation and survival at low oxygen levels. CACs, circulating angiogenic cells

angiogenic response *in vitro* [79], while in diabetic mice, inhibition of the formation of AGEs can restore ischaemia-induced angiogenesis in peripheral limbs [80]. The importance of methylglyoxal in key regulatory events is illustrated by the observation that the reduction of hypoxia-induced SDF-1, CXCR4 and VEGF production under high glucose conditions depends on the increased availability of methylglyoxal [43, 61, 81]. Indeed, overexpression of *GLO1*, which encodes glyoxalase 1, the rate-limiting enzyme in the detoxification of methylglyoxal, prevents the reduction of CXCR4 and VEGF levels observed in those conditions [61, 81]. Consistently, silencing of *GLO1* mimicked the methylglyoxal-induced effects on VEGF production [81].

These and other observations strongly suggest that the cell and tissue dysfunction associated with many diabetic complications is related, at least in part, to the loss of the cellular response to hypoxia. However, the molecular mechanisms underlying this dysfunction are still poorly understood.

Impairment of HIF-1 regulation in diabetes

Since HIF-1 is the master regulator of the cellular response to hypoxia, it is not surprising that HIF-1 deregulation is directly associated with the loss of cellular adaptation to low oxygen in diabetes. Indeed, there is a large body of evidence supporting this hypothesis and showing that HIF-1α is destabilised at low oxygen levels by high glucose concentrations. For example, Catrina et al. showed that high glucose decreases hypoxia-induced stabilisation and function of HIF-1α in human dermal fibroblasts and human dermal microvascular endothelial cells in culture. This destabilisation was not prevented by a prolyl hydroxylase inhibitor (ethyl 3,4-dihydroxybenzoate or EDBH) [67], suggesting that other non-canonical mechanisms may be involved in the regulation of HIF-1α protein turnover in

the presence of high glucose. In addition, HIF-1α production was found to be impaired during healing of large cutaneous wounds in young *db/db* mice and upregulation of HIF-1α by gene-based therapy was shown to accelerate wound healing and angiogenesis in this model [63, 68]. Moreover, levels of HIF-1α were found to be decreased in biopsies from foot ulcers of diabetic patients as compared with venous ulcers that share the same hypoxic environment but are not exposed to hyperglycaemia [67]. Downregulation of HIF-1 in response to hyperglycaemia also appears to account for the decreased arteriogenic response triggered by myocardial ischaemia in diabetic patients [82, 83]. In rats, myocardial infarct size increases in response to hyperglycaemia and is associated with reduced production of the HIF-1α protein [84]. As mentioned above, endothelial dysfunction in diabetes is related to impairment of hypoxia-induced production of eNOS, SDF-1, CXCR4 and VEGF. This impairment can presumably be ascribed to destabilisation of HIF-1α, since overexpression of *Hif-1α* normalises VEGF levels, improves development of myocardial capillary network and inhibits cardiomyocyte hypertrophy and cardiac fibrosis following myocardial injury [85]. In addition, increased expression or stabilisation of HIF-1α is critical to improve wound healing [59, 63, 68] and it enhances the vascular response to critical limb ischaemia in diabetic mice. This mechanism appears to involve an increase in limb perfusion and function, an increase in the number of circulating EPCs, vessel density and luminal area and a decrease in tissue necrosis [57].

Of significance, we and others have shown that impairment of the HIF-1 pathway under high glucose conditions may result from the increased availability of methylglyoxal. Indeed, *GLO1* overexpression is able to stabilise HIF-1α [43], as well as augment HIF-1 transactivation in response to hypoxia and high glucose levels [61]. In addition, under these conditions, *Glo1* overexpression was found to

increase levels of eNOS, VEGF, CXCR4 and SDF-1 [61], which might contribute to increased mobilisation of EPCs and improve the ischaemia-induced neovascularisation.

Other important findings include the observation that, compared with non-diabetic patients, patients with type 2 diabetes have decreased *HIF-1β* mRNA levels in pancreatic islets, suggesting that changes in the function of HIF-1 can contribute to the development of diabetes [86]. A human *HIF-1α* genetic polymorphism that results in P582S is associated both with type 2 diabetes [87, 88] and the absence of coronary collaterals in patients with ischaemic heart disease [89]. These observations highlight a critical link between diabetes, the HIF-1 pathway and endothelial dysfunction.

Despite the vast number of studies supporting a role for high glucose in HIF-1 destabilisation, it should also be noted that some reports have shown activation of HIF-1 in response to hyperglycaemia. For example, both mesenglial cells treated with high glucose [90] and glomerular cells from mice with streptozotocin-induced diabetes [91] showed increased production of HIF-1α and increased expression of hypoxia-induced genes, including *Vegf* and *Glut1*. It was further shown that glucose upregulates HIF-1α levels in primary cortical neurons exposed to hypoxia [92]. However, it should be noted that in some of these studies cells were treated with high levels of glucose for short periods of time, ranging from 3–5 h [92] to 48 h [90], which might explain the apparent discrepancy between these results and those of studies in which longer periods of incubation with high glucose were used. Additionally, one should not dismiss the fact that different animal models and different cell culture systems may produce different effects on HIF-1 activation following treatment with high glucose.

Proposed models for the loss of the cellular response to hypoxia in diabetes

Although the molecular mechanisms that underlie impairment of HIF-1 in diabetes remain poorly understood, some recent studies envision pathways whereby diabetes may lead to the downregulation of HIF-1. For example, Gurtner and collaborators reported two different mechanisms, both relying on the effect of increased availability of methylglyoxal in diabetes [61, 77]. Indeed, the authors showed that methylglyoxal modifies HIF-1α in hypoxic mouse dermal fibroblasts on two specific residues, arginine 17 and arginine 23, both of which belong to the basic helix-loop-helix domain that is critical for the interaction with HIF-1β and formation of an active heterodimer [61]. These modifications consistently reduced HIF-1 heterodimer formation (Fig. 3) and *Glo1* overexpression prevented this impairment, emphasising the role of methylglyoxal in the loss of the

cellular response to hypoxia in diabetes [61]. In a more recent study, the same group suggested that high glucose decreases the interaction between p300 and HIF-1α as a result of increased modification of p300 by methylglyoxal (Fig. 3). Mutation of arginine 354 of p300 completely prevented high-glucose-induced methylglyoxal modification of p300 and restored the interaction with HIF-1α [77]. The authors noted that methylglyoxal-induced modification of HIF-1α did not impair HIF-1α–p300 binding; however, a decrease in VEGF production was still observed, suggesting that impairment of associations between both HIF-1α–HIF-1β and HIF-1α–p300 might underlie the diabetes-induced defect in HIF-1 transcriptional activity [77]. The authors further observed that the iron chelator DFO improves HIF-1α–p300 binding and augments HIF-1 activity and VEGF production at high glucose levels, by preventing p300 modification by methylglyoxal via a mechanism dependent on the decreased production of reactive oxygen species [77]. Dimethyloxalylglycine (DMOG), an oxoglutarate analogue known to be a potent inhibitor of PHDs, did not show the same effects as DFO, suggesting that DFO-induced effects are not likely to be dependent on PHDs and to influence HIF-1α protein stability. Alternatively, DFO appears to normalise the high glucose-induced defect in HIF-mediated transactivation, by a mechanism dependent on the decreased production of reactive oxygen species. The physiological significance of this mechanism is indicated by the observation that DFO enhances wound healing and neovascularisation in diabetic mice [77].

A recent study by our group proposes a different mechanism for the regulation of HIF-1 under high glucose and hypoxic conditions, which also relies on methylglyoxal-induced modifications. We showed that methylglyoxal is capable of inducing modifications on HIF-1α (such as the formation of methylglyoxal-derived hydroimidazolone 1, also referred to as MG-H1, adducts), leading to the increased association of HIF-1α with the molecular chaperones HSP40 and HSP70. These molecular chaperones subsequently recruit CHIP, which induces the polyubiquitination of HIF-1α and its degradation [43] (Fig. 3). This mechanism of degradation appears to be mostly dependent on the proteasome, although other proteolytic pathways might also be involved in the degradation of methylglyoxal-modified HIF-1α. Canonically, CHIP has a key role in protein quality control by inducing ubiquitination of damaged proteins. The ability of CHIP to ubiquitinate HIF-1α under these conditions unravels an unanticipated role for CHIP in the loss of the cellular response to hypoxia under high glucose conditions such as diabetes. Interestingly, CHIP was also found to induce HIF-1α proteasomal degradation by a mechanism dependent on HSP70 in response to prolonged hypoxia [42]. Hypoxia is well known to increase the glycolytic rate and, most likely, the generation of reactive by-products of glycolysis, such as
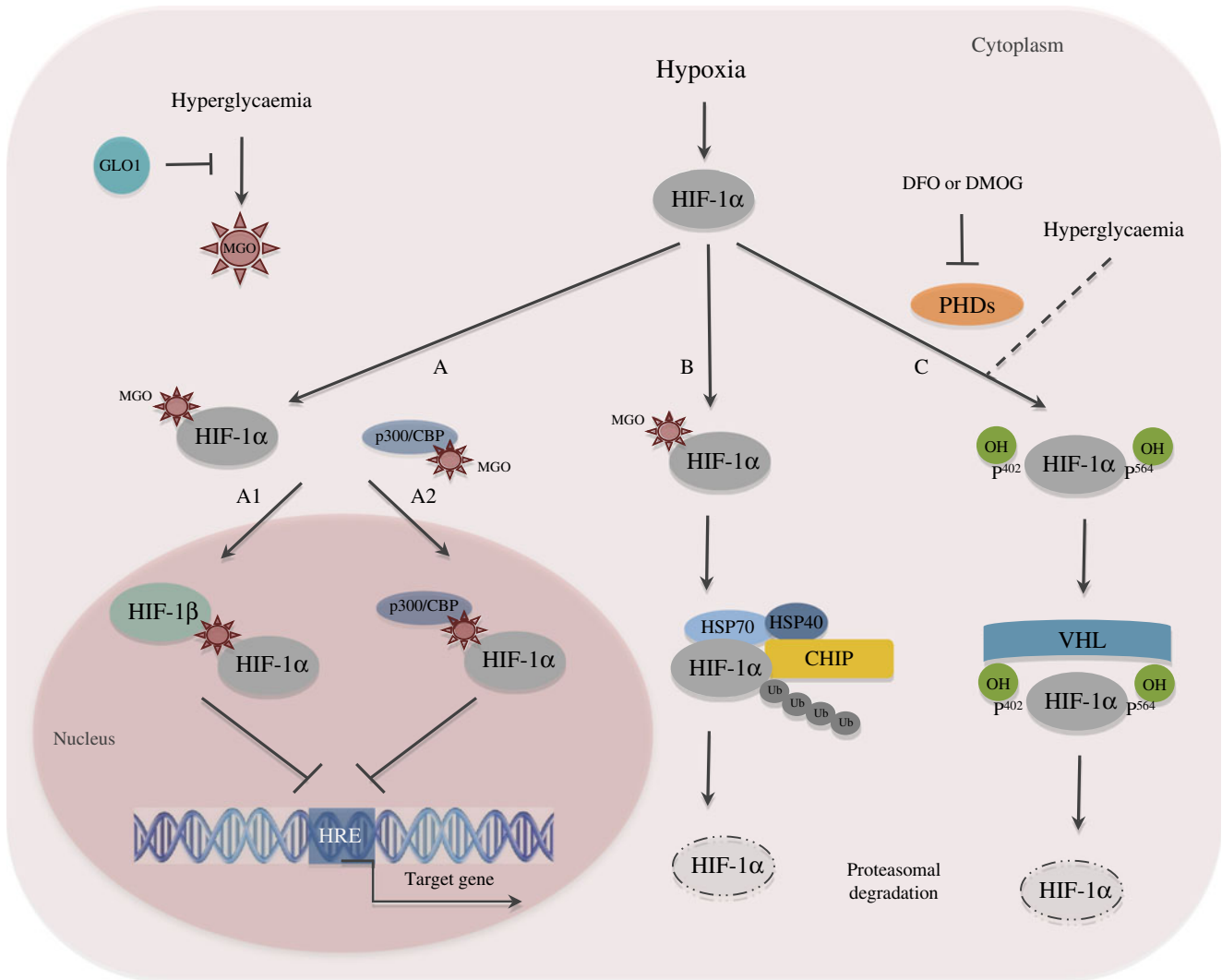
Fig. 3 Schematic representation of models proposed for the impairment of HIF-1 regulation in diabetic conditions. Hypoxia and hyperglycaemia are important features of most of diabetic complications. Three different models have been proposed to explain the loss of the cellular response to hypoxia and impairment of HIF-1 regulation in diabetes. In model A it is suggested that increased production of methylglyoxal (MGO) under high glucose conditions impairs the transcriptional activity of HIF-1, through mechanisms that involve methylglyoxal-induced modification of HIF-1α (model A1) [61] and of the HIF-1 co-activator p300 (model A2) [77]. Modification of HIF-1α leads to inhibition of HIF-1α–HIF-1β interaction, while p300 modification impairs p300–HIF-1α association, both changes culminating in decreased activity of the HIF-1 transcription factor. In model B it is proposed that methylglyoxal-induced modification of HIF-1α leads to increased interaction of HIF-1α with the molecular chaperones HSP40/HSP70, which subsequently leads to recruitment of CHIP that induces HIF-1α polyubiquitination and degradation by the proteasome [43]. Both models A and B rely on the increased production of methylglyoxal at high glucose levels, and inhibition of this increase by GLO1 appears to prevent the decreases in the activity of HIF-1 and stability of HIF-1α under high glucose and hypoxic conditions. In model C it is suggested that high glucose induces HIF-1α destabilisation by a mechanism dependent on VHL and HIF hydroxylases that is inhibited by DFO and DMOG [59], which are well known inhibitors of HIF-1α hydroxylation. Both models B and C rely on the increased degradation of HIF-1α under high glucose conditions

methylglyoxal. Thus, it is tempting to speculate that perhaps the CHIP-dependent degradation of HIF-1α under prolonged hypoxic conditions is associated with an increased availability of methylglyoxal. We further observed that *GLO1* overexpression was able to prevent methylglyoxal- and high glucose-induced destabilisation of HIF-1α under hypoxic conditions [43]. Furthermore, *GLO1* overexpression prevents the methylglyoxal-induced decrease of VEGF levels and silencing of GLO1 mimics the effects of methylglyoxal on the release of VEGF [81]. These data were obtained using cell culture systems and require physiological validation in animal models of diabetes, opening new avenues for future studies.

It is interesting to note that the two models proposed by Gurtner et al. and our CHIP-dependent model of HIF-1

deregulation are not inconsistent. Indeed, it is plausible that the decreased interaction between HIF-1α–p300 and HIF-1α–HIF-1β in the presence of methylglyoxal increases the amounts of modified and monomeric HIF-1α that are available to undergo degradation through a CHIP-mediated pathway. This hypothesis has not been confirmed nor addressed before, although it appears to be a promising possibility.

Other studies have suggested a role for HIF hydroxylases in impairment of the HIF-mediated cellular response to hypoxia in hyperglycaemia. Indeed, Catrina and collaborators found that inhibition of hydroxylases by DMOG or DFO prevents the repressive effects of high glucose levels on HIF-1α protein stability and activity and the expression of HIF-1 target genes [59]. This preventive effect was ablated in renal carcinoma cells producing functionally inactive VHL, suggesting a role for VHL in the destabilisation of HIF-1α at high glucose levels. Furthermore, local inhibition of HIF hydroxylases by DMOG and DFO improved the wound healing process in *db/db* diabetic animals and increased the stability of HIF-1α (Fig. 3). However, stabilisation of HIF-1α by DMOG and DFO in wounds of *db/db* mice was only partial compared with that in controls [59], suggesting that other mechanisms are likely to be involved in the destabilisation of HIF-1α under high glucose conditions.

These and other studies clearly show that further investigation is needed to clarify the mechanisms implicated in HIF-1 deregulation in diabetes. Some discrepancies may be due to cell- and tissue-specific regulatory mechanisms and further experimentation with animal models is required to corroborate the majority of data gathered in cell culture systems.

Conclusions and future directions

This review summarises some of the most recent developments accomplished in the area of the loss of the cellular response to hypoxia and impairment of HIF-1 regulation in diabetes. It has been clearly established that HIF-1α stability and function are compromised by high glucose concentrations and a few molecular models have been proposed to explain this impairment. Some of these models rely on methylglyoxal-induced modifications on HIF-1α or other components of the HIF-1 regulation pathway, which appear to compromise both the protein stability and function [43, 61]. Other groups have suggested a role for VHL and enzymatic hydroxylation in HIF-1α destabilisation at high glucose levels [59], which led to the hypothesis that therapeutic approaches based on the use of hydroxylase inhibitors might prevent many of the maladaptive responses derived from impairment of HIF-1 regulation by hyperglycaemia. Other therapeutic opportunities might be based on the overexpression of *GLO1* (to increase detoxification

of methylglyoxal and, as a consequence, to increase the stability and function of HIF-1α) [43, 61, 77, 81], inhibition or silencing of CHIP (to diminish the degradation of HIF-1α modified by methylglyoxal) [43] or overexpression of the gene encoding superoxide dismutase or other enzymes that may favour the decreased production of reactive oxygen species in diabetic tissues (to prevent methylglyoxal-induced modification of p300) [61]. Additionally, it should be noted that insulin can stimulate *HIF-1α* expression through a signalling pathway dependent on the activation of PI3K and the target of rapamycin (TOR) [93]. This could be of great significance since administration of insulin may ameliorate diabetic complications arising from a poor cellular response to low oxygen levels.

Unfortunately some of the approaches described above may be subject to limitations. For example, silencing or inhibition of CHIP may compromise several crucial cellular pathways and does not reverse the loss of function associated with direct modification of HIF-1α induced by methylglyoxal. Additionally, GLO1 function appears to be compromised in diabetes, since GLO1 is a GSH-dependent enzyme (reviewed in [94]) and GSH levels are decreased in diabetic tissues [95]. Thus, *GLO1* overexpression would probably require a concomitant strategy to increase GSH levels in cells. On the other hand, insulin, for example, was found to exacerbate the breakdown of the blood–retinal barrier via upregulation of HIF-1α and VEGF levels [96], which may lead to the worsening of diabetic retinopathy.

Taken together, these data indicate that further biological, pharmacological and clinical work is needed to better understand the mechanisms and molecular players involved in the loss of the cellular response to low oxygen in diabetes. These may contribute to the development of new therapeutic approaches for the treatment of diabetic complications associated with hypoxia.

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