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HIFs in hypoxic regulation of the extracellular matrix: focus on little-known player HIF-3

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Abstract: The structural and associated molecules of the extracellular matrix (ECM) complex is an important component of the local milieu of cells, both for maintaining their functions and homeostasis. It is a dynamic structure that is finely tuned to changes in the microenvironment. One of these factors is hypoxia, which can arise in tissues due to physiological or pathological effects. As a result of the hypoxic effect, the properties of the ECM are significantly modified, stiffness increases, the balance between degradation and synthesis of structural proteins shifts, and the deposition of biologically active mediators' changes. Hypoxia-inducible factors (HIFs) contribute significantly to the modification of the properties of the ECM under hypoxia. Among the three HIF isoforms, HIF-1 is the most studied, with numerous identified target genes encoding proteins that participate in all matrisome compartments. Much less is known about HIF-2 and HIF-3. In the context of the effects of hypoxia on the matrisome, HIF-3 isoform is of particular interest. Unlike the first two isoforms, transiently expressed during the first hours of hypoxia, HIF-3 is activated after around 24 h of exposure to hypoxia and persists for a longer period. In addition, its transcription is more pronounced under hypoxia than are HIFs 1 and 2. HIF-3, rather than the first two isoforms, is possibly responsible for the changes that occur in matrisome during prolonged hypoxic conditions. This review attempts to summarize the available data on the involvement of HIF-3 in the matrisome modification under hypoxia.

Introduction

The extracellular matrix (ECM) is a complex macromolecular structure that provides mechanical support to cells and actively regulates tissue physiology. The term "matrisome" was introduced to cover both the ECM structural and ECM-associated molecules [1]. The structural components of the core matrisome present: collagens, glycoproteins, proteoglycans, and glycosaminoglycans. Matrisome-associated molecules comprise regulatory proteins that take part in ECM remodeling, such as enzymes and their inhibitors; deposited biologically active molecules, including cytokines, growth factors, etc., as well as matrisome-associated molecules that are in charge of regulating the tissue microenvironment and modifying the biochemical properties of the matrix [1,2]. The components of the matrisome work cohesively to maintain a three-dimensional extracellular environment and deliver precise mechanical, structural, and biological guidance. Each tissue possesses a matrisome with its distinct composition and topology, resulting from the permanently changing biochemical and biophysical crosstalk between different cellular and extracellular components [3–5].

Changes in matrisome structure and functions are frequently observed in a range of pathological conditions often associated with oxygen deprivation (hypoxia). The role of reduced oxygen levels in ECM metabolism has been studied primarily in the context of vascular disease and tumor progression [6–8]. The adaptation to O_2 deprivation results in increased production, alignment, and stiffness of ECM structures. This increase is necessary to activate integrins [9–11], enhance expansion [12], and improve cell survival [13,14]. In particular, collagen I accumulation leads to an increase in tissue fibrosis [15–18], adhesive properties [9,19], and support of cell migration, thus promoting tumor invasion [13,20].

In 2019, the Nobel Prize in physiology or medicine was awarded jointly to W. G. Kaelin Jr, P. J. Ratcliffe, and G. L. Semenza "for their discoveries of how cells sense and adapt to oxygen availability" [21]. The principal signaling pathways of the cellular response to O_2 deprivation have

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been demonstrated to be triggered by a family of the hypoxia-inducible factors (HIFs) represented by three isoforms (HIF-1, -2, -3). The genes encoding matrisome proteins are widely represented among the targets of HIF-1 [22,23]. However, there are blind spots in this story related to the other isoforms, HIF-2 and HIF-3. Very little information has been accumulated on the latter one of these. Several studies suggest the existence of complex mechanisms in the mutual regulation of expression between HIF-1α, HIF-2α, and HIF-3α, including HIF-3α-mediated suppression of the expression of the first two factors [24–27]. This could indicate a potential shift in transcription activity towards HIF-3. This review will focus on the mechanisms of crosstalk between HIF-3 and the other HIF isoforms and the contribution of this to the regulation of matrisome components during cellular adaptation to hypoxia.

Hypoxia-inducible factor-mediated regulation of the cellular response to hypoxia

Hypoxia is one of the most important microenvironmental factors significantly affecting cellular metabolism. The O_2 levels *in vivo* typically range from 0.5% to 14%, depending on the type of tissue [28]. Thus, the O_2 concentration in the medullary niche or lymphoid organs is low (ranging from 0.5% to 4.5%) [29], but it reaches high values of up to 14% in kidney tissue [30]. Low tissue O_2 levels are physiologically normal for tissues and termed “physioxia”, or “physiological hypoxia”. These concentrations are appropriate for the cells and do not require adaptation. However, for a number of reasons, pathological conditions can occur that are accompanied by oxygen deprivation, such as myocardial infarction, stroke, tumors, chronic obstructive pulmonary disease, and other ischemic conditions [23,31,32]. Such hypoxic events can lead to adaptive or pathological outcomes, depending on the duration and degree of oxygen deprivation.

HIFs are the master regulators of cellular response to oxygen deprivation, triggering the expression of hundreds of target genes. The discovery of HIF-1 and its control mechanisms marked a significant milestone in studying cell adaptation to hypoxia. Currently, a significant body of knowledge about HIF-1, its regulation and targets has been accumulated, and two other isoforms, HIF-2 and HIF-3, have been discovered. HIFs are heterodimeric proteins consisting of HIF-1α, HIF-2α, or HIF-3α subunit and β-subunit (also known as aryl hydrocarbon receptor nuclear translocator (ARNT)) for each isoform, accordingly: HIF-1β (ARNT) and HIF-2β (ARNT2), while HIF-3β (ARNTL), is classified as a class II basic-helix-loop-helix-Per/Arnt/Sim (bHLH-PAS) factor [33]. The ARNT is expressed constitutively, while the HIF-1α, HIF-2α, and HIF-3α subunits are oxygen-sensitive. Under normoxic conditions, the α-subunit undergoes hydroxylation by the corresponding prolyl hydroxylases (PHD-1, -2, or -3), acquiring a hydroxyl group. This makes possible the binding of von Hippel-Lindau factor (VHL), which is a signal for ubiquitination and subsequent proteasomal degradation. Thus, when oxygen is in sufficient supply, degradation of the α-subunit occurs, and PHD acts as an oxygen sensor [23]. Among most HIF-3α variants, only one proline can be hydroxylated in ODD domains, making them less susceptible to PHD-mediated degradation [34,35]. Later, another oxygen sensor factor inhibiting HIF (FIH) was discovered, which hydroxylates an asparagine residue in the transactivation domain of the HIF-α subunit, thereby blocking the binding of the coactivator proteins p300 and the cAMP response element-binding protein (CREB) [36]. HIF-1α and HIF-2α contain asparagine residues that are hydroxylated by FIH in the C-terminal transactivation domain (C-TAD). As for HIF-3α, it does not include C-TAD [33,34]. Under hypoxia, hydroxylation slows down because PHD and FIH require oxygen for their activity. Therefore, the α-subunit accumulates and translocates to the nucleus. There, it dimerizes with the β-subunit to bind to the coactivator proteins p300 and CBP [23]. Then, binding to hypoxia responsive element (HRE) and activation of transcription of the target genes occurs. Most HRE sites can be recognized by both HIF-1 and HIF-2, indicating that they share common target genes. However, the differences between the N-TAD domains of HIF-1α and HIF-2α support the selective binding of HREs, providing the specificity of target genes for each HIF. Distinct yet very similar HRE sequences were identified for these two HIFs. All these genes showed no difference in their distribution patterns of HIF-1 and HIF-2 HREs. This supports the view that HIF-1/HIF-2 common targets are recognized in a non-competitive and non-compensatory manner in the same regions. A specific motive for HIF-3 has not been proposed to date. Genes with promoter regions enriched in HIF-1 motifs were induced by acute hypoxia and maintained their activity during prolonged hypoxic exposure. Genes with promoter regions enriched in HIF-2 motifs are activated only by prolonged hypoxia. Thus, HIF-1 and HIF-2 do not appear to compete for the same HREs [33,37]. Some of the HIF-targeted genes encoding matrisome-related molecules are presented in Fig. 1.

In addition, the activity of other cue players in the canonical hypoxic pathway may indirectly affect HIFs. For example, prolyl hydroxylase activity may be regulated by several intracellular factors, including reactive oxygen species (ROS) [38], which are in turn negatively modulated by mitochondrial deacetylase sirtuin-3 (SIRT3) [39]. The M2 isoform of pyruvate kinase (PKM2) acts as a coactivator of HIF-1α and HIF-2α. PKM2 expression is known to be induced by HIF-1α. PKM2 directly binds to HIF-1α and HIF-2α, enhances their binding to HREs, and promotes the transactivation of hypoxia-inducible genes by HIF-1 and HIF-2 [40].

Numerous microRNAs participate in HIF regulation and can either repress or activate expression affecting other HIF isoforms or regulatory genes. For instance, hypoxia-induced hsa-miR-147 has been shown to suppress the *HIF3A* transcript, thus supporting HIF-1 activity in human cervical cancer cells (HeLa) [41]. In turn, direct binding of HIF-3α with miR-630 promoter induces miR-630 expression. A positive feedback loop has been found between HIF-3α-mediated overexpression of miR-630 and elevation of HIF-3α, while the expression of HIF-1α was suppressed by miR-630 [42].

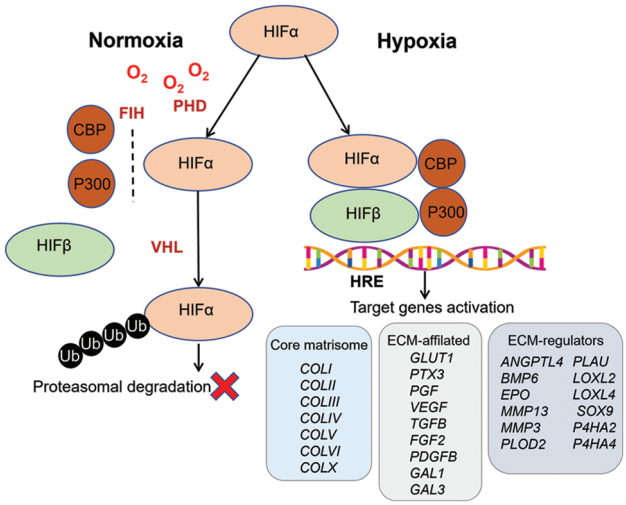

FIGURE 1. Hypoxia-inducible factors (HIFs) in the regulation of cellular response to hypoxia. HIFs are heterodimeric proteins consisting of oxygen-sensitive HIF-1α, HIF-2α, or HIF-3α subunits and a constitutively expressed HIF-1β subunit. Under normoxic conditions, the α-subunit undergoes hydroxylation by the corresponding prolyl hydroxylases (PHD), binding by von Hippel-Lindau factor (VHL), and subsequent proteasomal degradation. Factor inhibiting HIF (FIH) hydroxylates the C-terminal transactivation domain (C-TAD), blocking the binding of the coactivator proteins p300 and the CREB-binding protein (CBP, where CREB stands for cAMP response element-binding protein). Under hypoxia, hydroxylation slows down because PHD and FIH require oxygen for their activity. Therefore, the α-subunit accumulates and translocates to the nucleus. There it dimerizes with the β-subunit and binds to the coactivator proteins p300 and CBP. Then, this complex binds to hypoxia responsive element (HRE) and consequently, the activation of transcription of the target genes occurs, among other genes encoding matrisome-related molecules.

The contribution of hypoxia-inducible factor-1 and -2 in the regulation of physiological processes under hypoxia

Several genes essential for the adaptation and survival of cells at low-oxygen environments are targeted by the HIF factors [23]. More than a thousand HIF-dependent genes have integral roles in a variety of biological processes, including angiogenesis [43–45], cellular metabolism [43,46,47], cell signaling [48,49], cell cycle regulation [50,51], cellular response to damage [44,49,52], stemness [53,54], and tumor progression [52,54,55]. In particular, HIF controls the transcription of vascular endothelial growth factor (VEGF)—the main driver of tissue vascularization. This achieves increased supply as a result of the newly-developed blood vessels [43]. HIF-1 regulates the expression of genes encoding stromal cell-derived factor 1 (SDF-1) [56,57], integrin a4 [10], and matrix metalloproteinases (MMP) [58,59], all of which have had an impact on cell migration. HIF-1α has been shown to be associated with microRNAs that regulate signaling pathways under hypoxia, for instance, with miR-210, which supports the survival and proliferation of stromal progenitors under reduced oxygen levels [60,61]. HIF-1α is also involved in mediating the effects of hypoxia on differentiation [62,63].

HIF-1 and HIF-2 are thought to have overlapping roles, but it cannot be claimed that they are completely redundant [64]. The variations exist in the target genes. The genes encoding heme oxygenase-1, phosphofructokinase, lactate dehydrogenase, and phosphoglycerate kinase were targeted by HIF-1 only [65,66]. Conversely, HIF-2 but not HIF-1, controlled the expression of stemness genes such as those for octamer binding transcription factor (*OCT*)-*3/4* and matrix metalloproteinases 2 and 3 [67,68]. Only HIF-2 has been shown to regulate the solute carrier family 11 member 2 gene (*SLC11A2*), which encodes the iron transporter protein of the divalent metal transporter 1 (DMT1) [69]. The contribution of HIF-1 and HIF-2 in oxygen sensing is uneven: HIF-1 is the more important for survival, as its knockout leads to developmental arrest and lethality [70], whereas HIF-2 knockout mice survived despite the severe defects of vascularization [71]. HIF-1 is a regulator of the response to hypoxia in all eukaryotic cells, whereas HIF-2 is only present in vertebrates. Although HIF-2 is present in many tissues during embryonic development, its expression is less prominent in adult cells, and it has become more specific for well-vascularized tissues, for example, kidney, placenta, and lungs [72,73]. It is considered that erythropoietin (EPO)-a master regulator of erythropoiesis, is controlled by HIFs. Some papers have described HIF-1 involvement in *EPO* upregulation [23,74]. Meanwhile, there are data indicating that HIF-2 participates in *EPO* upregulation as well. In the murine liver, *EPO* has been found to be upregulated by HIF-2, but not by HIF-1 [75]. Studies in mice with postnatal deletion of *HIF2A* and in Hep3B cells using siRNA have shown that HIF-2, but not HIF-1, plays a key role in the regulation of EPO [76]. Another study reported that it could be regulated by not only HIF-1 and HIF-2, but also by HIF-3 [77].

Other transcription factors may also contribute to HIF-1/2-mediated stimulation of target gene activity under hypoxia. These factors differ depending on the isoform. For instance, signal transducer and activator of transcription 3 (STAT3) binds to promoters of HIF-1 target genes, while upstream transcription factor 2 preferentially binds to promoters of HIF-2 target genes. Transcription of the target gene is then triggered by the formation of complexes with the corresponding alpha subunit [78].

There are some time-course differences in the expression of these factors. For example, the expression of *HIF1A* is transient and decreases after a few hours of activity. The transcription of *HIF2A* is more prolonged [79–81].

As mentioned above, of all the factors of the HIF family, the third isoform, HIF-3, is by far the least studied. Many questions still need to be answered about its influence on the regulation of biological processes in the cell, its involvement in pathological processes in the body, and its interaction with the first two isoforms.

The structure of hypoxia-inducible factor-3 and its role in the regulation of the cellular response to hypoxia

The HIF-3α subunit was first described as a novel α-class hypoxia-inducible factor in mice [82]. This isoform was later found in humans [83]. Human HIF-3α is located on

chromosome 19q13.13 13.2 (12), which differs from the location of HIF-1α (14q21 24) and HIF-2α (2p16 21) [84]. The structure of HIF-3α differs from the a-subunits of the other two HIFs. For example, HIF-3α contains a leucine zipper (LZIP) that allows for protein-protein interactions. In addition, it has a transactivation domain (TAD) site at its N-terminus only, whereas HIF-1α/2α has these sites on both termini (N and C), and has many alternative splice variants (10 in humans) (Fig. 2). The long splice variants include the entire set of domains, although sometimes with the exception of LZIP. The short splice variants may lack one or more domains.

The presence/absence of certain domains in HIF-3α molecules appears to determine their functions. For example, short splice variants lacking the basic-helix-loop-helix (bHLH), Per/Arnt/Sim (PAS), and PAS-associated COOH-terminal (PAC) domains that are responsible for oxygen sensing and dimerization with the β-subunit, are independent of oxygen levels and therefore perform different functions. Furthermore, various splice variants are often differentially expressed and regulated depending on the tissues concern, the developmental stages, and other factors [26,83]. Certain splice variants have been well studied, while data on some others, mostly the short splice variants, are rather scarce. Moreover, not all studies dealing with the HIF-3α target genes and their biological effects aimed to identify the specific splice variants involved.

Multiple splice variants are not unique to HIF-3α. HIF-1α and HIF-2α also undergo alternative splicing. According to the National Center for Biotechnology Information (NCBI) and Ensemble databases, there may be 12 different variants of the HIF-1α and HIF-2α transcripts, of which the short variants are also poorly studied [35]. Furthermore, the NCBI database indicates there are 19 splice variants predicted for human HIF-3α, 10 of which have been experimentally confirmed.

Pasanen et al. investigated the expression of HIF-3α splice variants in various adult tissues and cell lines. They could not detect HIF-3α3, HIF-3α5, and HIF-3α6. The HIF-3α1, 2, 8, and 9 variants were expressed in all tissues except the placenta. They detected HIF-3α10 in the heart, brain, skeletal muscle, kidney, and pancreas tissues. HIF-3α4 was expressed in the spleen and brain, while HIF-3α7 was detected in the heart, placenta, lung, liver, and skeletal muscles [26]. A similar distribution pattern of the HIF-3α splice variant in tissues has been demonstrated in adult mice [82]. In addition, the expression of inhibitory PAS domain protein (IPAS), which is thought to be a negative regulator of hypoxia-inducible genes, has been detected in the retina and Purkinje cells [85]. Neonatal embryonic PAS protein (NEPAS), associated with embryonic and neonatal development, is highly expressed in embryonic heart, lung, brain, kidney, and liver tissues [24].

In other vertebrates, zebrafish, HIF-3α1 expression has been described in adult ovaries, kidneys, testes, and gills. However, in other tissues, high levels of HIF-3α1 were detected only during embryogenesis [24]. Zebrafish HIF-3α2 is lacking the bHLH, PAS, and PAC domains. It is expressed in both embryonic and adult tissues and could inhibit canonical Wnt through b-catenin binding [86].

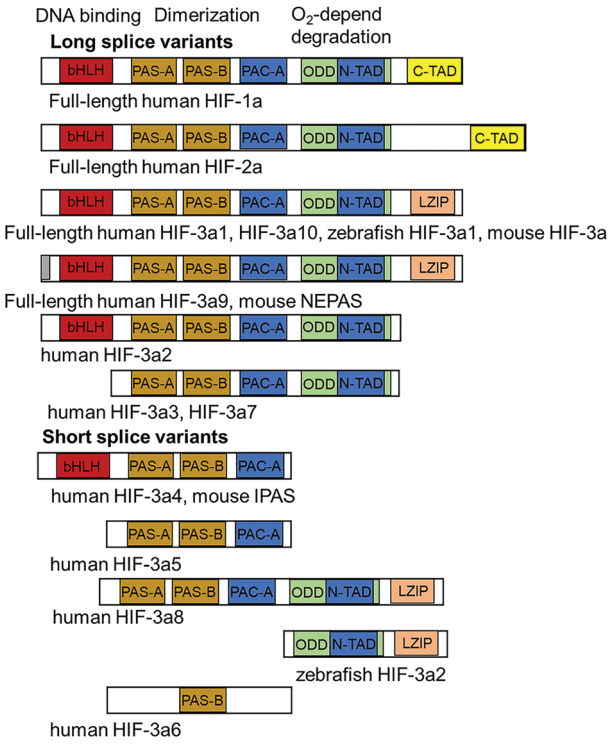

FIGURE 2. The structure of hypoxia-inducible factors (HIFs). HIF-1/2 isoforms include a basic-helix-loop-helix (bHLH) located at the N-terminus, which is responsible for DNA binding; PAS (Per/Arnt/Sim)-associated COOH-terminal domain (PAC-A). The PAS-A and PAS-B regions dimerize with the bHLH region of the HIF β-subunit (ARNT). Next, the oxygen-dependent degradation domain (ODD) is responsible for oxygen-dependent degradation and transactivation of domain (TAD) at the N-terminus and C-terminus [83]. Long splice variants HIF-3α include a bHLH located at the N-terminus, PAC-A, PAS-A, and PAS-B. Next, the ODD domain and TAD are at the N-terminus [34,83,84]. The C-terminus contains the leucine zipper (LZIP). Both the LZIP and TAD are involved in protein-protein interactions. Among the long splice variants, there are the full-length human HIF-3α1 and the HIF-3α10, the zebrafish HIF-3α1, and the mouse HIF-3α isoforms. Human HIF-3α10 was recently discovered and differs from HIF-3α1 by the presence of a 210-bp intron 1 [26]. Full-length human HIF-3α9 and mouse neonatal embryonic PAS protein (NEPAS) have the same structure as the splice variants described above, except for a difference in some amino acids at the N-terminus. The splice variant human HIF-3α2 lacks the LZIP domain, while human HIF-3α3 and HIF-3α7 also lack the bHLH domain. The short splice variants of human HIF-3α4 and mouse inhibitory PAS domain protein (IPAS) do not include LZIP, ODD and TAD. Human HIF-3α5 lacks bHLH in addition to those mentioned, while human HIF-3α8 lacks only bHLH compared with the full-length variant. There are even shorter splice variants: zebrafish HIF-3α2 carrying only the ODD, TAD, and LZIP domains, and human HIF-3α6 with a PAS-B domain [34].

HIF-3α is thought to be less involved in carcinogenesis than HIF-1α. Panasen et al. have tested a significant number of tumor cell lines with HIF-1α expression, but only a few have demonstrated the presence of HIF-3α splice variants [26].

Thus, the data from vertebrate studies show a large variation in the distribution of HIF-3α splice variant expression depending on the tissue and developmental stage

concerned. HIF-3α differs significantly from the HIF-1α/HIF-2α isoforms in its structure and expression in tissues. The regulatory activity of HIF-3α and its involvement in cell adaptation to the microenvironment have been studied in much less detail, but a number of fundamental differences from the first two HIFs have been revealed.

Similarly to HIF-1 and HIF-2, HIF-3 directly regulates transcription by binding to HREs at promoter sites of target genes. For instance, under 1% hypoxia, chromatin immunoprecipitation analysis has detected HIF-3α2 binding associated with canonical HRE in the promoter regions of *EPO* and *ANGPTL4* in Hep3B hepatoma cells [77] and in the promoter regions of *REDD1, MLP3C*, and *SQRDL* in zebrafish embryos provoking the upregulation of above genes (Fig. 3). In addition, HIFs can recruit other factors, including signaling pathway regulators, to improve HIF binding and further transcription. HIF-3α has been found to be a stimulator of osteosarcoma proliferation through lysine demethylase 3A (KDM3A)-dependent demethylation of the SOX9 promoter. Under hypoxic conditions, *KDM3A* expression was upregulated by HIF-3α, and *KDM3A* occupied the SRY box transcription factor 9 (SOX9) gene promoter region by H3 lysine 9 demethylation (H3K9me2) [87]. Overexpression of *HIF3A* in pulmonary artery smooth muscle cells was followed by elevated expression of phosphatidylinositol 3 kinase (PI3K) and protein kinase B (Akt) proteins associated with cell migration, survival, and inactivation of apoptosis [88,89]. In transgenic mice, full-length human HIF-3α bound a conserved HRE site in the Sox2 promoter and weakly transactivated a reporter construct containing the Sox2 promoter region. In this way, HIF-3α significantly contributed to mouse lung development [90]. An increase in the phosphorylation of HIF-3α-downstream signal transducer Janus kinase and activator of transcription (Jak/STAT) was demonstrated in cytotrophoblast [91]. HIF-3α regulates cell proliferation and cell apoptosis in extravillous cytotrophoblast, promoting the Fms like tyrosine kinase receptor-1 expression and phosphorylation levels of Jak2 and STAT3 (Fig. 3).

The target genes of HIF-3 may differ significantly from those of HIF-1 and HIF-2. For example, in rat hepatocytes, *HIF3A* overexpression caused an increase in the expression of insulin-like growth factor (IGF) binding protein 1 (IGFBP-1), whereas *HIF1A* upregulation had no such effect [92]. HIF-3α knockdown experiments in zebrafish have shown that upregulation of the insulin-like growth factor binding protein 1 (*IGFBP-1B*), sulfide quinone reductase-like (*SQRDL*), microcin LB (*MCLB*), and zona pellucida glycoprotein 3d tandem duplicate 2 (*ZP3V2*) genes was induced predominantly by the splice variant HIF-3α1 [93]. Whole genome analysis of zebrafish embryos with stable production of HIF-1α and HIF-3α revealed 690 HIF-1α-dependent upregulated genes. Among these, only 155 genes were upregulated by HIF-3α. In addition, there were only 97 overlapping genes, suggesting the existence of genes regulated by only one of the HIF isoforms. For example, only HIF-3α regulated genes encoding molecules of Janus kinase-STAT and the nucleotide oligomerization domain (NOD)-like receptor signaling pathways, whereas HIF-1α mediated both VEGF and mitogen-activated protein kinase (MAPK) signaling [93].

HIF-1α target genes can be inhibited by short splice variants of HIF-3α. In particular, HIF-3α4 has been shown to suppress the HIF-1α-mediated gene encoding glucose transporter 1 (*GLUT1*) and *VEGF* expression in human cancer cells [94]. Similar data were obtained in endothelial and vascular smooth muscle cells when the HIF-3α2 splice variant suppressed the expression of HIF-1-dependent genes encoding VEGF-α and Enolase2 [27]. In addition, the activity of HIF-1α or HIF-2α can be inhibited directly by some splice variants of HIF-3α. For example, in mice, NEPAS binds competitively to the HIF-1β subunit, thereby limiting its availability for HIF-1α and HIF-2α [24]. Direct binding of long human HIF-3α or IPAS to HIF-1α and HIF-2α has been shown to prevent the translocation of the latter into the nucleus [25,85,94]. HIF-3α1 has been shown to downregulate HIF-1 and HIF-2-mediated genes in renal cancer cells. This was due to the competition of HIF-3α

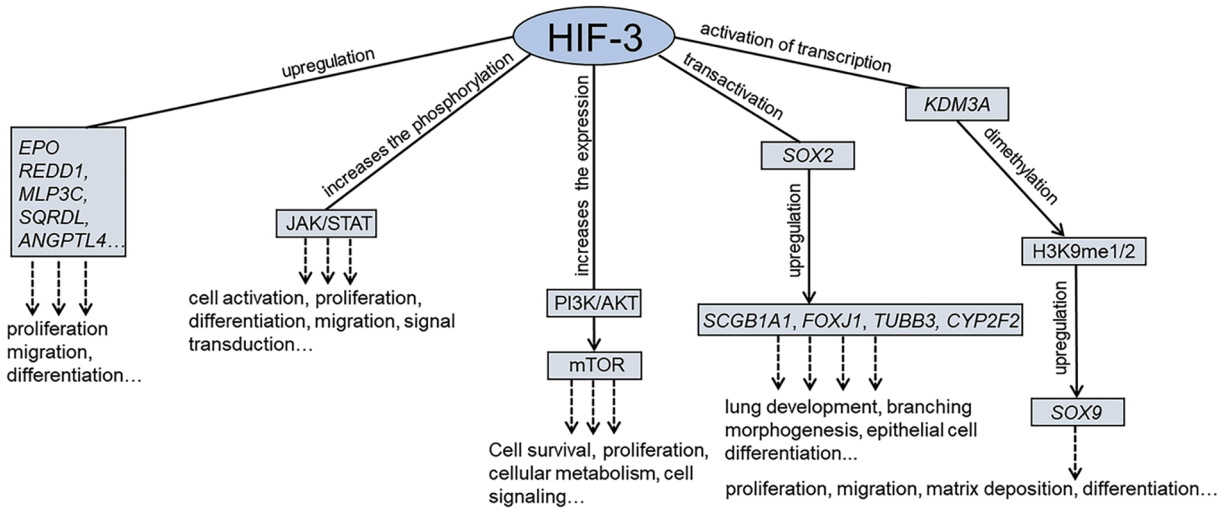

FIGURE 3. The molecular mechanisms regulated by hypoxia-inducible factors-3 (HIF-3). Similarly to HIF-1 and HIF-2, HIF-3 directly regulates transcription by binding to hypoxia-responsive elements (HREs) at promoter sites of target genes. In addition, HIFs can recruit other factors, including signaling pathway regulators, to improve HIF binding and further transcription.

with the other isoforms for binding to HIF-1β. As a result, the levels of HIF-1α and HIF-2α decreased, and the transcription of the HIF-1 and HIF-2 target genes was downregulated [83]. Using live-imaging, Kim et al. demonstrated that HIF-3α2/HIF-3α4 splice variants function as negative regulators on the heterodimer formation of HIF-2α with HIF-1β in human prostate cancer cells [95].

The HIF-3α-dependent inhibition of other isoforms can be suggested to not be due to their antagonism but is a step in the adaptation to prolonged hypoxic exposure. Indeed, HIF-α isoforms have been shown to have different time points of activation and time courses of expression. In our laboratory, after exposure of human multipotent mesenchymal stromal cells (MSCs) under 5% O_2, *HIF1A* upregulation was detected for up to 4 h and *HIF2A*–upregulation – for up to 12 h. *HIF3A* was upregulated between 12 and 24 h [79]. In lung epithelial cells at 1% O_2, *HIF1A* expression was increased after 30 min but decreased between 8 and 16 h, while *HIF3A* transcript levels peaked at 8–16 h [96]. In human endothelial cells under 1% O_2, *HIF1A* upregulation was seen up to 4 h during hypoxic exposure, while *HIF3A* was upregulated after 4 h, with transcription reaching a maximum after 48 h of exposure [97]. In human bladder smooth muscle cells, the upregulation of *HIF1A* and *HIF2A* was detected after 2 h of hypoxia but then rapidly declined, while *HIF3A* was upregulated after 48 h, and remained at high levels for up to 72 h [98].

Both in human cells *in vitro* and in rodents *in vivo*, it has been shown that HIF-1α and HIF-2α alone, or together, can stimulate HIF-3α expression [26,27,85,99]. These data suggested a transition of expression activity from transient *HIF1A* and *HIF2A* to the prolonged overexpression of *HIF3A* [26,80]. This mechanism may be critical for cell survival under prolonged hypoxia. Thus, HIF-1 responds first under hypoxia and then "turns on" *HIF3A* expression in the later steps of adaptation. Bartoszewska et al. have identified that microRNA miR-429 plays a major role in HIF-1a-dependent control of *HIF3A* expression [80]. In particular, in human endothelial cells, acute hypoxia has been characterized by the accumulation of HIF-1α, while HIF-2α and HIF-3α have been accumulated in the chronic state [100,101].

The oxygen levels required to maintain high expression of O_2-inducible HIF-1α and HIF-3α may differ. In lung epithelial cells, *HIF3A* was maximally upregulated at 3% O_2, whereas *HIF1A* was maximally upregulated at 1% O_2 [96]. In addition, HIF-3α responds to hypoxia (or its mimetic $CoCl_2$) and also to inflammatory cytokines and some growth factors. In MSCs, interleukin 6 (IL-6), tumor necrosis factor (TNF-α), monocyte chemoattractant protein-1 (MCP-1), epidermal growth factor (EGF), and VEGF stimulate HIF-3α both under normoxic and hypoxic conditions, while HIF-1α expression was increased only under hypoxia [102].

In conclusion, HIF-3α differs from the other HIF isoforms in its time course and duration of expression, target genes, and tissue specificity. This may be important for adaptation to prolonged hypoxia and the inflammatory microenvironment that often accompanies hypoxia.

The structural and functional compartments of matrisome under hypoxia

Reduced O_2 levels impact many processes in cells and, as a consequence, the properties of secreted ECM and ECM-associated mediators. The effects of hypoxia on the matrisome are mainly studied in the context of the tumor microenvironment and ischemic tissues. Changes in matrix stiffness, ECM deposition, and degradation, upon exposure to hypoxia, are largely mediated by HIF factors, as have been detected in different *in vitro* studies.

It has been clearly demonstrated that resident fibroblasts from different tissues deposit increased levels of collagen I under hypoxic conditions [103–105]. The growth factors, such as the transforming growth factor (TGF-β), actively participate in ECM deposition. In fibroblasts, HIF-1α and HIF-2α have been shown to be involved in TGF-β pathway activation [106,107]. HIF-1α also participates in regulating transcription of the prolyl 4-hydroxylase subunit alpha 1 (*P4HA1*) and alpha 2 (*P4HA2*) genes encoding prolyl hydroxylase expression in fibroblasts. When cultured at hypoxia, human corneal fibroblasts have been shown to enhance the deposition of several matrix molecules, including collagens I, V, and VI, plus fibronectin [12]. Pleomorphic adenoma SM-AP1 and SM-AP4 cell lines showed increased production of perlecan and fibronectin under hypoxia. Under normobaric hypoxia *in vivo*, the deposition of collagens I, III, and IV, plus fibronectin in rat lungs and of fibronectin, fibulins, and aggrecan in mouse vascular endothelial cells have also been demonstrated [108,109].

After 48 h exposure at 1% O_2, proteomic profiling of MSCs revealed decreased levels of collagen molecules (COL1a2, COL1a1, and COL3a1) and of ECM organization-related proteins including fibulin 2 (FBLN2), fibulin 1 (FBN1), COL14a2, laminin alpha 5 (LAMA5), ECM protein 1 (ECM1), and of secreted protein acidic and cysteine-rich (SPARC). Reactome annotation of the proteins highlighted pathways related to glycosaminoglycan metabolism, the organization of ECM, including elastin fibers, and the degradation of chondroitin sulfate or dermatan sulfate [110]. Mass spectroscopic analysis of conditioned medium from MSCs after 24 h at 1% O_2 showed an increased accumulation of proteins involved in the tensile strength, three-dimensional folding, and mechanical stability of collagen fibrils [111].

After hypoxic exposure, the alteration of post-translational modification of collagens that resulted in enhanced alignment and stiffness of ECM fibers has been described [13,112,113]. Increased expression of prolyl 4-hydroxylase subunit alpha 1 (P4HA1) and procollagen-lysine,2-oxoglutarate 5-dioxygenase l (PLOD1), which are key enzymes for intracellular modification of procollagen molecules, has been reported in various cell types, including tumor and stromal cells [13,114,115]. In addition, hypoxia-dependent extracellular modification of fibrillary collagens through increase the production of lysyl oxidase (LOX) and the lysyl oxidase-like enzymes (LOXL-2 and LOXL-4) ensure the formation of cross-linking between collagenous fibers [13,116]. These thin, aligned fibrils promote cell

migration. Furthermore, the upregulation of genes encoding the MMP-2 and MMP-9, as well as of urokinase-type plasminogen activator (PLAU) and urokinase-type plasminogen activator receptor (UPAR), has been described [117–119].

These results demonstrate significant modulation of all matrisome compartments under hypoxic exposure. Our next step will be the analysis of the data with respect to the involvement of hypoxia-inducible factors in these changes.

Hypoxia-inducible factor-1 and -2-mediated regulation of the matrisome

Extracellular matrix deposition and stiffness

Silencing of HIF-1α transcription with siRNA confirmed that core matrix proteins are indeed upregulated by HIF-1α [120]. HIF-1α also participates in regulating *P4HA*1 and *P4HA2*, thereby controlling prolyl hydroxylase expression in fibroblasts. The prolyl hydroxylation of collagen leads to its stabilization and prevents its degradation, which in turn promotes its deposition. The lysyl hydroxylases involved in this process are encoded by the *PLOD1*, *PLOD2*, and *PLOD3* [121,122]; *PLOD2* is regulated by HIF-1α. As a result of *PLOD2* activation, procollagen α-chains form a triple helix and are secreted into the ECM, due to the hydroxylation of proline and lysine residues [123,124]. LOXs mediate further cross-linking between collagens and elastin, increasing the stiffness of the matrix [125]. As HIF-1α targets, the *LOXL2* and *LOXL4* genes are upregulated by hypoxia [116,126].

Degradation of extracellular matrix

In addition to increasing stiffness through collagen modifications and cross-linking, hypoxia also affects ECM degradation. This process occurs through the action of proteinases, particularly the MMP family, which makes a significant contribution [127]. MMPs, a family of zinc-dependent enzymes that cleave ECM components, are classified into several types, including gelatinases, stromelysins, collagenases, and cell membrane-associated MMPs, each with specificity for different substrates [7].

Under hypoxia, the upregulation of *MMP14*, which encodes enzyme cleaves collagen types I, II, and III, has been demonstrated. This gene was shown to be a target for both HIF-1 and HIF-2 [128,129]. *MMP2* and *MMP9*, whose encoding enzymes degrade type IV collagen, were also upregulated under hypoxia. They were targeted by HIF-1 only [117,130]. MMP3 expression was increased in rheumatoid arthritis synoviocytes transfected with a plasmid that expresses HIF-1α. Here, MMP-3 expression was suppressed upon siRNA-mediated silencing the HIF-1α [131]. Hypoxia can upregulate the *PLAUR* gene in breast cancer cells and the intestinal epithelium. This regulation is mediated by the HIF-1α [118]. The activation of the corresponding receptor produced an effect similar to that of MMP, resulting in the degradation of several ECM components, including the basal membrane [7].

Matrix-associated molecules

A number of ECM-associated molecules and deposited soluble factors have been found to be targets of HIF-1 or HIF-2. For transforming growth factor (TGF-β), HIF-1α and HIF-2α involvement in TGF-β pathway activation has been demonstrated in fibroblasts and human choriocarcinoma cells cultured under hypoxic conditions [103,132]. HIF-1α-dependent induction of basic fibroblast growth factor (bFGF) under hypoxia has been shown in human endothelial cells [133], as well as induction of bFGF and platelet-derived growth factor-BB (PDGF-BB) in breast cancer cells [134]. VEGF, one of the growth factors that is deposited in the ECM, is a well-known target of HIF-1 and is significantly upregulated under hypoxia [135,136]. Galectins are matrix-associated molecules with immunomodulatory properties. They can also be induced by HIF-1α. Under hypoxia, there are data on the beneficial effects of HIFs on the expression of galectin 1 in human cardiomyocytes and colorectal cancer cells [137,138] and of galectin 3–in human pancreatic cells and canine mammary tumors [139,140].

The cited data indicate that matrisome changes under hypoxia have implications for the physiological state of tissues. Thus, HIF-1-mediated deposition of collagen I and the increased stiffness of the ECM are the main processes leading to fibrosis [7]. Overexpression of HIF-1α induces the progression of fibrotic alterations in primary mouse tubular epithelial cells and in the development of adipose tissue fibrosis in transgenic mice [15,16]. By contrast, HIF-1α-deficient mice showed reduced liver fibrosis [17]. Increased stiffness and concomitant degradation of the ECM contribute significantly to developing osteoarthritis (OA) [141]. Excessive deposition of ECM by tumor-activated fibroblasts and an increase in ECM stiffness, as well as HIF-1α-mediated increase in the cell surface expression of integrins [109,142], facilitate cell migration [7]. In part, this can be related to HIF-1-dependent *PLOD2* activity. Blocking *PLOD2* decreased the ability of cells to migrate and reduced lung metastasis in mouse models of sarcoma [115]. Thus, numerous data sets have confirmed that both HIF-1 and HIF-2 regulate the expression of genes encoding proteins in all the compartments of the matrisome in a similar manner.

Hypoxia-inducible factor-3-mediated regulation of the matrisome proteins

Based on the available research, the HIF-1- and HIF-2-dependent changes of the matrisome could be reasonably considered as acute response to hypoxic stress. Their expression was upregulated shortly after the O_2 deprivation and was sustained for only a relatively short period (a few hours). The involvement of HIF-3 as a long-lasting transcription factor in the prolonged adaptation of cells to moderate hypoxia is of significant interest. However, the data concerning this are scarce and quite contradictory.

As already mentioned, *HIF1A* and *HIF2A* were transiently upregulated in cultured human bladder smooth muscle cells after 2 h of exposure to hypoxia, while *HIF3A* was upregulated after 72 h [98]. The protein levels of HIF-1α and HIF-3α were significantly elevated after 2 and 72 h, respectively. Consistent with these changes, upregulation of genes encoding matrisome components and an increase in the levels of these molecules were observed. The study found that genes encoding collagens I, II, III, and IV plus

fibronectin, aggrecan, connective tissue growth factor (CTGF), similar to mothers against decapentaplegic mad (SMAD) 2, and SMAD 3, as well as metallopeptidase inhibitor 1 (TIMP-1) were all upregulated. Additionally, there was a time-dependent increase in total collagenous proteins. The inflammatory cytokines IL-1β, IL-6, TNF-α (mRNA) and TGF-β (protein and mRNA) showed a time-dependent increase in expression, while the anti-inflammatory IL-10 decreased after 72 h. Furthermore, *VEGF* was upregulated. The signs of smooth muscle cell dedifferentiation, such as an increase in α-smooth muscle actin (αSMA), vimentin, and desmin, could also indicate an increase in the matrix-producing activity of the cells. Therefore, a positive correlation was found in the time-course dynamics of the expression of matrisome proteins, inflammatory cytokines, and HIF-3α expression. The authors hypothesized that HIF-3α may stimulate matrix deposition and subsequent fibrotic changes in the bladder wall [98,143].

However, these studies only show a positive correlation between HIF-3 and the matrisome proteins. They do not provide evidence that HIF-3 regulates the gene expression of these proteins. Li et al. examined the involvement of HIF-3α in ECM production in cultured human chondrocytes from healthy and OA samples by implementing the *HIF3A* knockdown with miR 210 [141]. The development of OA is directly related to changes in the ECM, these being manifested by increased collagen deposition and cartilage degeneration. In cultured OA chondrocytes, the expression of cartilage-specific collagen type II alpha 1 chain (*COL2A1*) was downregulated, while the expression of fibrosis-associated collagen type X alpha 1 chain (*COL10A1*) and *MMP13* were upregulated compared to chondrocytes from healthy tissue. These data suggest increased cartilage degradation in OA. MiR-210-mediated knockdown of *HIF3A* resulted in enhanced proliferation of OA chondrocytes and the upregulation of chondrocyte-specific *COL2A1*, whereas the expression of *COL10A1* and *MMP13* mRNA was downregulated. This indicates the involvement of HIF-3 in the pathogenesis of OA through its effects on ECM components [141].

However, the inverse role of HIF-3α in the development of OA has been demonstrated in rat lipopolysaccharide (LPS)-induced OA. In chondrocytes, HIF-3α was shown to be involved in the inhibition of inflammation and matrix degradation via the FOXO1/PRG4/HIF-3α axis. LPS decreased the expression of HIF-3α, the level of forkhead box O1 (FOXO1) and proteoglycan 4 (PRG4), whereas uncu-51 like autophagy activating kinase 1, beclin, and microtubule-associated protein 1 light chain 3 proteins, which are involved in the development of OA, increased. The secretion of inflammatory IL-1β and TNF-α was also stimulated. Upregulation of *HIF3A* led to degradation of the ECM, increased production of MMP13, of a disintegrin, of metalloproteinase with thrombospondin motifs (ADAMTS5) and collagen type II but decreased the IL-1β and TNF-α levels. Thus, this study concluded that HIF-3α contributes to the degradation of excessively deposited ECM in OA, stimulates chondro-specific collagen II, but reduces the levels of inflammatory cytokines [144]. Comparing their results with those of Li et al. [141], the authors hypothesized that the disparate effects may be due to the use of different experimental models and the fact that in rodents, the mechanisms involving HIF-3α may work differently than in humans.

In the mouse model, upregulation of *HIF3A* resulted in the downregulation of *VEGF*, *COL101A1* and *MMP13* [145]. However, these findings are not consistent with data on collagen I expression in human cells [141] and the upregulation of *MMP13* in rats [144]. Therefore, the effects of HIF-3α on the matrisome are not entirely clear, and the explanation for the differences is likely more complex than just species-dependent mechanisms.

The data on the HIF-3α effect on the expression of certain proteins of the matrisome or factors deposited therein are summarized in Table 1.

TABLE 1

HIF-3α and matrisome proteins

Gene/ Protein	HIF3-a effect	Experimental model	Splice variant	Reference
Core matrisome				
COL101A1	Downregulation	C3H10T1/2 mouse cell line 1% hypoxia for 8 h	Not determined	[145]
COL2A1	Downregulation	Human osteoarthritic and normal chondrocytes	Not determined	[141]
COLII	Decrease	Rat BMMSCs	Not determined	[146]
Collagen I	Increase	Lung tissue of mice exposed to cigarette smoke	Not determined	[147]
Collagen II	Increase	Rat chondrocytes, LPS-induced osteoarthritis	Not determined	[144]
ECM-regulators				
ANGPTL4	Upregulation	Human Hep3B hepatoma cells, overexpression *HIF3A*, 1% O_2 24 h	HIF-3α2 long	[77]
BMP6	Upregulation	Human Hep3B hepatoma cells, overexpression *HIF3A*, 1% O_2 24 h	HIF-3α2 long	[77]
ADAMTS5	Increase	Rat chondrocytes, LPS-induced osteoarthritis	Not determined	[144]
EPO	Upregulation	Human Hep3B hepatoma cells, overexpression *HIF3A*, 1% O_2 24 h	HIF-3α2 long	[77]

(Continued)

Table 1 (continued)

Gene/ Protein	HIF3-a effect	Experimental model	Splice variant	Reference
MMP13	Downregulation	C3H10T1/2 mouse cell line 1% hypoxia for 8 h	Not determined	[145]
MMP13	Upregulation	Human osteoarthritic and normal chondrocytes	Not determined	[141]
MMP13	Negative correlation	Human MSCs and articular chondrocytes from healthy and osteoarthritic tissues	HIF-3a4, HIF-3a7, HIF-3a8, HIF-3a9	[148]
MMP13	Increase	Rat chondrocytes, LPS-induced osteoarthritis	Not determined	[144]
SOX9	Decrease	Rat BMMSCs	Not determined	[146]
ECM-associated				
GLUT1	Downregulation	Human renal cell carcinoma cells and osteosarcoma cells, overexpressed *HIF3A*, 1% O_2, 4 h	HIF-3a4	[94]
IGFBP1A	Upregulation	Zebrafish embryos (HIF-3a mRNA forced expression)	Not determined	[93]
IGFBP1B	Upregulation	Zebrafish embryos (HIF-3a mRNA forced expression)	Not determined	[93]
IL-1β	Decrease	Rat chondrocytes, LPS-induced osteoarthritis	Not determined	[144]
PTX3	Upregulation	Human Hep3B hepatoma cells, overexpression *HIF3A*, 1% O_2 24 h	HIF-3α2 long	[77]
PGF	Upregulation	Human Hep3B hepatoma cells, overexpression *HIF3A*, 1% O_2 24 h	HIF-3α2 long	[77]
REDD1	Upregulation	Zebrafish embryos (HIF-3a mRNA forced expression)	Not determined	[93]
TNF-α	Decrease	Rat chondrocytes, LPS-induced osteoarthritis	Not determined	[144]
VEGF	Downregulation	C3H10T1/2 mouse cell line 1% hypoxia for 8 h	Not determined	[145]
VEGFA	Downregulation	HUVEC, 0.5% O_2, 48 h	HIF-3a2, HIF-3a3,	[27]
VEGF	Downregulation	Human renal cell carcinoma cells and osteosarcoma cells, overexpressed *HIF3A*, 1% O_2, 4 h	HIF-3a4	[94]
VEGF	No change	HUVEC, UtMVEC, HPAEC, HCAEC, HIAEC, HPASMC, HMVEC-D and HMVEC-L, 0,9% O_2, 48 h	HIF-3a2	[100]
VEGF	Decrease	Tnni2K175del mutation mice	Not determined	[149]

Note: ADAMTS5, metalloproteinase with thrombospondin motifs; ANGPTL4, angiopoietin-like protein 4; COL101A1, collagen type X alpha 1 chain; COL2A1, collagen type II alpha 1 chain; COLII, collagen II; ECM, extracellular matrix; EPO, erythropoietin; GLUT1, glucose transporter 1; HIF, hypoxia inducible factor; IGFBP1A, insulin-like growth factor binding protein 1a; IL-1β, interleukin-1β; MMP, matrix metalloproteinase; PGF, placental growth factor; PTX3, pentraxin 3; REDD1, regulated in development and DNA damage response 1; SOX9, SRY-related HMG box 9; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

In Hep3B cells, overexpression of the splice variant HIF-3α2 led to upregulation of genes encoding bone morphogenetic protein 6 (*BMP6*), thereby enhancing deposition and mineralization of the ECM [150] and overexpression of *EPO* and placental growth factor (*PGF*). These growth factors are known to induce the secretion of matrix metalloproteinases [151,152], angiopoietin-like protein (ANGPTL) involved in matrix degradation and inflammation [153], and pentraxin 3 (PTX3), which has anti-inflammatory effects and provides interconnection between matrix structural components [154]. Silencing of HIF-3a with siRNA downregulated the corresponding genes [77]. The relationship between HIF-3α and the expression of αSMA, a marker of myofibroblasts, was investigated by [155]. Myofibroblasts respond to TGF-β with extra collagen production, leading to fibrotic changes. The authors concluded that HIF-3α could potentially be a negative regulator of αSMA expression, thereby attenuating ECM production [155]. Blocking HIF-3α with miR-210 was shown to upregulate collagen type II (*COL2*) and SRY-related HMG box 9 (*SOX9*), whose encoded proteins are responsible for maintaining both matrix structure and matrix deposition [146]. Conversely, miR-210 inhibitors reduced ECM deposition. After co-transfection of rat stromal cells with miR-210 inhibitors and HIF-3α siRNA or the transfection of miR-210 inhibitors, there was an increase in the expression of the COLII and SOX9 proteins in the case of co-transfection. Thus, HIF-3α negatively regulates COLII and SOX9 production and decreases ECM deposition, promoting its overall degradation [146].

In human chondrocytes, HIF-3α expression was negatively correlated with the transcription of *COL10A1* and *MMP13*, markers of ECM hypertrophy. These data were confirmed in HIF-3α knockdown experiments, which showed increased *COL10A1* and *MMP13* expression [148]. In the lungs of mice exposed to cigarette smoke, the deposition of collagen I was HIF-3α-dependent [147]. In a rat model of partial bladder outlet obstruction (pBOO), a concomitant increase in the expression of HIF-3α and collagen types I and III was interpreted as an indicator of fibrosis [156]. In the cartilage of $Tnni2^{K175del}$ mutant mice, HIF-3α was shown to negatively regulate the expression of *VEGF*, this protein being an ECM-associated growth factor involved in regeneration and vasculogenesis [149,157]. By contrast, HIF-3α4 overexpression in HepG2 and HEK293A cells provoked the downregulation of *VEGF* and *GLUT1*

[94,158]. In human umbilical vein endothelial cells (HUVEC), overexpressing HIF-3α2 and HIF-3α3 resulted in significant downregulation of hypoxia-induced *VEGFA*. When the transcription of HIF-3α was silenced by small hairpin RNA (shRNA), the transcription of *VEGFA* was restored [27]. In contrast to these data, *HIF3A* knockdown had no effect on *VEGFA* transcription in several endothelial lines [100].

In zebrafish embryos, forced expression of HIF-3α by injecting its mRNA accompanied the upregulation of *IGFBP1A* and *IGFBP1B* genes for the insulin-like growth factor-binding proteins that activate bioactive matrix molecules, further inducing matrix mineralization via IGF1 [159]. As well as being involved in matrix deposition, these genes are regulated both during development and by the DNA damage response 1 (*REDD1*) gene, thus potentiating fibronectin production [160]. Dominant-negative inhibition and knockdown experiments resulting in the downregulation of *HIF3A*, confirmed the precise effect of HIF-3α on the transcription of the above-mentioned genes [93].

To summarize, HIF-3-upregulation and the downregulation of collagen types I and II have been demonstrated under hypoxia. Increased transcription and synthesis of proteins involved in maintaining and remodeling matrix structures, such as those encoded by *PTX3*, *EPO*, and *PGF* have also been described. In addition, the expression of HIF-3α is tissue- and organ-specific, and does not provide a ubiquitous response to hypoxia [34]. Based on the above data, the variation in HIF-3 regulation may be species-dependent. Besides, the functional difference between the splice variants of HIF-3α provides a wide field for studying HIF-3-mediated regulation of the core matrisome and matrisome-associated molecules.

Conclusion

The response to hypoxia is manifested by significant metabolic changes in cells and their matrisome. Hypoxia, as an important microenvironmental cue, influences both the core matrisome and regulatory and matrix-associated molecules. HIFs are pivotal to cellular responses. The HIF-1 and HIF-2 isoforms regulate the matrisome in a similar manner: increasing matrix stiffness, promoting the accumulation of profibrotic collagens, and the deposition of biologically active mediators such as VEGF, TGF-β, bFGF, and PDGF-BB, besides accelerating ECM degradation due to increased metalloproteinase activity.

The available data reflect the ambiguity of the role of HIF-3 in matrix remodeling under hypoxia. Like HIF-1 and HIF-2, HIF-3 can govern profibrotic changes in matrisomes. HIF-3 upregulates the transcription of genes, such as *BMP6, COL1A1*, and *ANGPTL*, encoding molecules involved in ECM deposition and mineralization. Suppression of the myofibroblast phenotype and enhanced expression of genes encoding inflammatory molecules have also been demonstrated under HIF-3 control. HIF-3-mediated deposition of profibrotic proteins such as collagens type I or III was demonstrated.

Meanwhile, HIF-3 mediates antifibrotic effects by enhancing tissue-specific collagen II production and preventing OA. Additionally, it upregulates genes encoding molecules that contribute to the maintenance of the tissue-specific structure of the ECM, such as *PTX3, EPO, PGF*, and *COL2A1*. These observations are intriguing, and further study could open up wide prospects for treating diseases associated with pathological matrix changes, such as OA and pBOO.

The data presented here support the more intricate mechanism of HIF-3 involvement in matrisome regulation, considering factors such as oxygen levels, splice variant activities, species, and tissue sources. Future studies will focus on the accumulation of data and the identification of the signaling pathways that are involved in this regulation. The involvement of HIF-3 in adapting to prolonged hypoxia and supporting HIF-1 and HIF-2-initiated responses to acute oxygen deprivation broadens our perspective on regulating the matrisome response to hypoxic microenvironments.

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