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Hypoxia-driven pathways in bone development, regeneration and disease

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Hypoxia-driven pathways in bone development, regeneration and disease

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Abstract

Adaptation to hypoxia is a critical cellular event both in pathological settings, such as cancer and ischemia, and in normal development and differentiation. Oxygen is thought to be not only an indispensable metabolic substrate for a variety of in vivo enzymatic reactions including mitochondrial respiration, but also a key regulatory signal in tissue development and homeostasis by controlling a specific genetic program. Hypoxia-inducible transcription factors (HIFs) HIF-1 and HIF-2 are central mediators of the homeostatic response that enables cells to survive and differentiate in low-oxygen conditions. Genetically altered mice have identified important roles for HIF-1 and HIF-2 as well as vascular endothelial growth factor-A (VEGF)—a potent angiogenic factor and a downstream target of the HIF pathway—in the regulation of skeletal development, bone homeostasis and haematopoiesis. In this Review, we summarize the current knowledge of HIF signalling in cartilage, bone and haematopoiesis, and pay particular attention to the complex relationship between HIF and VEGF in these tissues based on data collected in animal models. The study of these models expands our understanding of the cell autonomous, paracrine and autocrine effects that mediate the homeostatic responses downstream of HIFs and VEGF. This knowledge can also be relevant for diseases like cancer and ischemia.

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Competing interests

The authors declare no competing interests.

Key points

- Oxygen levels regulate specific signalling cascades, such as the hypoxia-inducible factor (HIF) signalling pathway
- HIFs are essential mediators of the complex homeostatic responses that enable hypoxic cells to survive and differentiate
- VEGF is a downstream target of the HIF pathway and a potent angiogenic factor
- HIFs and VEGF have critical roles in skeletal development and bone homeostasis, as well as in haematopoiesis
- HIFs and VEGF are also crucial for bone regeneration and are involved in osteoarthritis and metastasis of tumours to bone

[H1] Introduction

The ability of a cell to adapt to hypoxic conditions is critical both in pathological settings, such as cancer and ischemia, and in normal development and differentiation. Oxygen is thought to be not only an indispensable metabolic substrate in a variety of enzymatic reactions *in vivo* including mitochondrial respiration, but also a key regulatory signal in tissue development and homeostasis. For example, during embryonic development, cellular differentiation as well as organ growth and final shape are thought to be modulated by oxygen gradients, which, at least in part, rely on the hypoxia-inducible factor (HIF) signalling pathway to mediate their effects.[1,2] The transcription factors HIF-1 and HIF-2 are central mediators of the homeostatic responses that enable hypoxic cells to survive and differentiate.[1,2] These proteins trigger a range of autonomous, autocrine, paracrine and endocrine effects with the overall goal of increasing oxygen delivery to tissues while decreasing their oxygen consumption.[3,4,5]

HIF-1[6-10] consists of two basic helix–loop–helix proteins of the PER–ARNT–SIM subfamily, HIF-1α and HIF-1β,[9–11] which are ubiquitously expressed.[12] HIF-1α is activated when oxygen levels drop to <5%, which is detected by a class of 2-oxoglutarate-dependent and Fe^{2+}-dependent prolyl hydroxylases (Figure 1).[13-18] By contrast, HIF-1β is constitutively expressed in an oxygen-independent manner. In tissues where the oxygen tension is >5%, the half-life of HIF-1α is very short (<5 min). However, under hypoxic conditions, HIF-1α protein accumulates, translocates to the nucleus, dimerizes with HIF-1β, and, upon recruitment of various transcriptional co-activators, binds to hypoxia response elements within the promoters of hypoxia-

responsive genes.[19] Of note, "normoxia" and "hypoxia" are relative concepts, depending on the tissues and/or organs of interest.

The HIF-2α homologue is regulated by oxygen tension in a similar way to HIF-1α and can also form a transcription complex with HIF-1β.[19] However, HIF-2α and HIF-1α have overlapping and unique biological functions.[9,19] For example, regulation of expression of enzymes of the glycolytic pathway is a unique HIF-1α function, whereas control of erythropoiesis *in vivo* appears to be specific to HIF-2α; last, modulation of angiogenesis is a common function of both HIF-1α and HIF-2α.[19] Interestingly, expression of either HIF-1α or HIF-2α can be controlled by growth factors in a hypoxia-independent manner.[20,21] The intracellular oxygen tension is, therefore, not the only upstream regulator of HIF-1α and HIF-2α.

Alongside their involvement in hypoxia adaptation, important roles have been identified for HIF-1α and HIF-2α in the regulation of bone development and homeostasis, as well as in haematopoiesis.[22,23] HIF-1α and HIF-2α have also been implicated in human regenerative processes and disorders; increased levels of at least one of the two proteins are clinically associated with fracture healing, osteoarthritis and metastasis of tumours to bone.[23] To date, more than 100 putative HIF target genes have been identified that are involved in a variety of biological processes,[23,24,25-28] such as anaerobic metabolism and angiogenesis.[27,29] Included amongst these is VEGF, the main angiogenic factor induced by the HIF signalling pathway, which has important roles in various physiological and pathological conditions.[30] VEGF is a homodimeric glycoprotein 45 kDa in size that belongs to the dimeric cysteine-knot growth factor superfamily.[30],[31] The *VEGF* gene encodes various differentially spliced protein isoforms: the three main ones in mice are

VEGF120, VEGF164, and VEGF188.[30],[31] In contrast to VEGF188, the soluble isoform VEGF120 does not bind to the extracellular matrix component heparan sulfate or the co-receptor neuropilin.[30],[31] VEGF164, however, is both soluble and able to bind heparan sulphate and neuropilin, which implies this isoform shares biological and biochemical properties with both VEGF120 and VEGF 188.[30],[31] In the mouse embryo very strict regulation of the levels of VEGF signalling is essential for normal angiogenesis.[32,33]

In this Review, we summarize the current knowledge of HIF signalling in cartilage, bone and haematopoiesis, and pay particular attention to the complex relationship between HIF and VEGF in these tissues based on data collected in animal models.

[H1] Cartilage and bone development

During embryonic development, the HIF signalling pathway has an essential role in coordinating organogenesis and angiogenesis. The growing tissues of the embryo rapidly deplete local oxygen and nutrient supplies provided via diffusion, which establishes oxygen gradients.[2] The hypoxic regions promote formation of blood vessels.[2] Skeletal development offers a paradigm of this concept. Most bones (including all the long bones of the axial and appendicular skeleton) develop in the embryo through intermediate cartilaginous templates, in a process called endochondral ossification.[34],[35] These templates direct the growth of endochondral bones, initially as cartilaginous anlagen that subsequently develop into the foetal growth plates, and are unique, avascular mesenchymal tissues that become hypoxic as they grow.[36] Chondrocytes are remarkably competent at surviving and differentiating in this hypoxic environment, at least in part by virtue of the actions of HIFs.[36] However, chondrocytes themselves also promote

localized vascularization at the periphery of the cartilage, and this process is required for the continued development and growth of bone.[30] In fact, the process of endochondral ossification is itself driven by vascularization, as terminally differentiated chondrocytes induce an angiogenic switch that coordinates the breakdown of cartilage and its controlled replacement by vascularized bone tissue.[37] Findings of the past decade collectively show that formation of all types of bone occurs in close spatial and temporal association with vascularization of the ossified tissue, a concept now termed angiogenic–osteogenic coupling.[38,39]

Endochondral ossification begins with mesenchymal cells condensing at the site of the future cartilage template; these cells then differentiate into chondrocytes. Chondrocytes in the initial cartilaginous moulds (and later in the foetal growth plates) synthesize a characteristic extracellular matrix that is rich in type II collagen. These cells are highly proliferative, piling up to form columnar layers of cells (Figure 2). The most distal cells of this layer stop dividing and differentiate into hypertrophic chondrocytes that produce type X collagen and mineralize their surrounding matrix. Proliferative chondrocytes are resistant to vascular invasion because of the presence of angiogenic inhibitors such as chondromudulin 1 and tenomodulin produced by these cells.[40] In mice, therefore, the centre of the cartilage mould becomes increasingly hypoxic as the foetal growth plate expands in the absence of blood vessels.[36,41] In contrast, the differentiation of chondrocytes to hypertrophic chondrocytes coincides with production of angiogenic stimuli (such as VEGF) by these cells and attraction of blood vessels.[30] Along with the invading blood vessels, osteoblast precursors and specialized osteoclasts called chondroclasts enter the region of the terminal hypertrophic chondrocytes and mediate

erosion of the underlying cartilage.[34,42] Differentiating osteoblasts lay down bone matrix, which is dominated by type I collagen, on top of the calcified matrix deposited by the hypertrophic chondrocytes.[34,42] Altogether, these events establish the primary ossification centre, which consists of the primitive bone and bone marrow cavity, in the central region of developing long bones.[34,42]

The conversion of avascular cartilage anlagen into highly vascularized bone and marrow tissues involves various vascularization processes.[34 35 30 37 42] Invasion by blood vessels from the surrounding perichondrium is the initial trigger for primary ossification.[34 35 30 37 42] Progressive capillary invasion at the metaphyseal border of the growth plate cartilage mediates further rapid bone lengthening.[34 35 30 37 42] Another blood vessel network overlies the avascular cartilage at the ends of the bones (epiphyses) and expands on the surface of the cartilage mass as it grows. [34 35 30 37 42] Postnatally, these vessels invade the epiphyseal cartilage and initiate formation of the secondary ossification centres, in each end of the long bones, as a prelude to the end of longitudinal bone growth.[37.41]

[H2] HIF and VEGF involvement

The crucial roles of HIF and VEGF in governing the survival of hypoxic cartilage, its invasion by blood vessels and the vascularization of growing endochondral bones have been elucidated in a number of studies over the past decade.[36, 37,22,23] Use of genetically modified mice has shed light on how HIF-1 and VEGF modulate endochondral bone development *in vivo*. Although much of the underlying mechanisms remain to be clarified, the functional features and interactions are beginning to be unravelled.

As a prime mediator of chondrogenesis, HIF-1α expression and function are necessary for the survival of chondrocytes in their hypoxic environment; conditional deletion of this protein in chondrocytes causes massive cell death in the inner, most hypoxic zones of developing growth plates.[36] The mechanism underlying this role of HIF-1α in cell survival is likely to involve a number of factors, but some evidence suggests that HIF-1α turns on genes that enable chondrocytes to switch to oxygen-sparing metabolic pathways.[1,43] As such, by activating anaerobic glycolysis in cartilage, HIF-1α prevents overconsumption of scarce oxygen in this challenged avascular tissue.[43] The oxygen tension, therefore, is held within a likely narrow though optimum range for hypoxic chondrocytes to survive and differentiate normally.[43]

In addition to its effects on metabolism, HIF-1α improves the efficiency of post-translational modifications of type II collagen, which is the main constituent of the cartilaginous matrix.[44] These modifications include hydroxylation of the collagen proline residues by a family of collagen prolyl-hydroxylases distinct from the family of prolyl-hydroxylases that hydroxylate HIFs. Proline hydroxylation is a critical step in the formation of the collagen triple helix.[44] Enhanced expression of these enzymes by hypoxia might explain the positive effect of HIF-1α on matrix accumulation by chondrocytes.[45] [44] Moreover, promoting the formation of an appropriately structured extracellular matrix might be one of the modalities by which HIF-1α ensures chondrocyte survival by modulating cell-matrix interactions.[45] [44]

A role for hypoxia and HIFs in chondrocyte proliferation and differentiation, as well as in joint development, has also been observed.[46,47] Conditional knockout of HIF-1α in the limb bud mesenchyme of mice indicated that HIF-1α expression is probably not required

for the initiation of mesenchyme condensation.[46,47] However, HIF-1α is critically involved in the timely differentiation of the mesenchymal cells into chondrocytes, via mechanisms that are still largely unknown, although regulation of SOX-9 (a principal transcription factor involved in chondrogenesis) might be involved.[48,46] In addition, lack of HIF-1α delays the terminal differentiation of chondrocytes, which is probably a downstream effect of delays in the initiation of chondrogenesis.[46,47] Notably, conditional HIF-1α -knockout mice also showed a striking impairment of joint development,[46,47] a defect that could also be related to SOX-9 because (at least *in vitro*) hypoxia increases matrix accumulation by chondrocytes of the articular surface, in a manner dependent on this transcription factor.[49] Finally, consistent with the notion that hypoxia leads to arrest of growth,[50] HIFs seem to negatively modulate chondrocyte proliferation.[36] Altogether, conditional knockout of HIF-1α in chondrocytes or limb bud mesenchyme led to dwarfism and marked shortening of the limbs.[36,47,46]

In contrast to HIF-1α -knockout, HIF-2α -knockout causes only a modest and transient delay in endochondral bone development.[51,21] Embryos heterozygous for a null allele at the locus encoding HIF-2α (*Epas1*$^{+/-}$) were mildly dwarfed, but this phenotype was no longer detectable by 2 weeks after birth in comparison with wild-type (*Epas1*$^{+/+}$) littermates.[21] Moreover, conditional deletion of HIF-2α in the mouse limb bud mesenchyme resulted in a mild and transient delay in endochondral bone development,[51] via mechanisms that are still largely unknown. Taken together, these findings indicate that HIF-1α is necessary for growth plate development, whereas HIF-2α is not essential.

VEGF is a classic target of HIFs and expressed, like HIF-1α, in the central hypoxic zone of the foetal growth plate, albeit at low levels.[41,52,53] Conditional knockout of *VEGF* in mouse chondrocytes or global inactivation of the soluble VEGF isoforms (VEGF120 and VEGF164) resulted in a phenotype characterized by cell death at the centre of the foetal growth plate that closely mimicked that observed in animals with HIF-1α -deficient growth plates.[41,53] *In vitro*, hypoxia increased VEGF accumulation in chondrocytes in a manner dependent on HIF-1α.[54,55] Hence, the survival-promoting functions of HIF-1α in hypoxic chondrocytes could potentially be mediated, fully or in part, via its downstream target VEGF. However, testing of this hypothesis revealed that the lethal effect of HIF-1α -knockout in chondrocytes could not be completely rescued by transgenic expression of VEGF164, which implied the involvement of VEGF-independent, cell-autonomous mechanisms.[43] Still, expression of VEGF164 in proliferating chondrocytes is required to ensure an adequate oxygen supply to the cartilage, which is achieved by inducing angiogenesis in the surrounding perichondrium.[43] Upregulation of *VEGF* expression in proliferating chondrocytes by hypoxia and/or HIF-1α, could thus be critical to enable appropriate vascularization of the perichondrium, through diffusion of the soluble VEGF isoforms produced by chondrocytes.[41,43]

In addition to regulating blood vessel formation in the soft tissue surrounding cartilage, VEGF also induces vascularization within the endochondral bones during skeletal development and growth.[56–57] VEGF is expressed at high levels by hypertrophic chondrocytes in the foetal growth plates, where it is critical for blood vessel invasion and replacement of cartilage by bone.[56–57] Osteoblasts and osteoclasts express several VEGF receptors and respond to VEGF signalling by enhanced recruitment, differentiation,

activity and/or survival (Figure 3).[30,37,58] These pleiotropic actions of VEGF on various cells in the bone environment might contribute to the tight coordination of vascularization, ossification and matrix resorption that is characteristically seen in endochondral bone development and growth.

[H1] Bone homeostasis

Adult bone continuously undergoes remodelling, which enables the maintenance of skeletal and mineral homeostasis.[73] The balance between bone formation by osteoblasts (of mesenchymal origin) and bone resorption by osteoclasts (of hematopoietic origin) is critical in bone remodelling and failure to coordinate these processes can result in either porotic or sclerotic bone.[59] Unlike cartilage, the bone marrow is highly vascularized, and although the role of this plentiful blood supply in bone modelling and remodelling is poorly defined, it is likely to go beyond being a mere source of nutrients.[38] Indeed, these blood vessels deliver both osteoclasts[60] and osteoblast precursors to the bone .[42,61]

Modulation of the HIF pathway in cells of the osteoblast lineage revealed that this pathway has important roles in bone formation (Figure 3).[62] Conditional loss of HIF-1α or HIF-2α in mouse osteoblasts caused a significant decrease in bone volume,[63] whereas increased levels of HIF-1α and HIF-2α resulting from knockout of the von Hippel–Lindau (VHL) tumour suppressor led to an augmentation of bone volume.[62] Notably, the changes in bone volume correlated positively with changes in bone vascularization and VEGF expression, suggesting a possible link between angiogenesis and osteoblastogenesis.[38] Specifically, bone pericytes could be the link between these processes as they reside in the blood vessel wall and are precursors to osteoblasts.[61]

Furthermore, in adult mice, conditional induction of VEGF expression in cells of the osteoblast lineage dramatically increased bone volume and caused aberrant vascularization.[64] Despite mounting evidence of an important role for angiogenesis in increasing bone volume via upregulation of the HIF pathway, autonomous cellular mechanisms downstream of HIF, such as regulation of anaerobic metabolism, might be important too since anaerobic metabolism is one of the main HIF targets as extensively discussed above.

[H1] Haematopoiesis

In adult mammals, haematopoiesis occurs almost exclusively in the bone marrow, which suggests that this environment regulates the process. Genetic studies in mice have shown that bone and the bone marrow are complex, dynamic microenvironments populated by multiple cell types, including hematopoietic stem cells (HSCs), which contribute to myelopoiesis and lymphopoiesis.[65,66] For example, some evidence indicates that osteoblasts regulate the number of HSCs as well as their differentiation along the lymphoid lineage by activating the Notch signalling pathway and secreting cytokines such as IL-7, respectively.[65] In addition, independently of their property to deliver osteoblast precursors, bone marrow blood vessels have been identified as a critical niche for HSC survival and differentiation.[66,67]

Despite its high degree of vascularization, bone marrow is relatively hypoxic compared to other adult tissues,[68,69] which is probably the result, at least in part, of oxygen consumption by the high number of hematopoietic cells that populate the bone marrow. Mathematical models have shown that a layer only three cells deep of myeloid progenitor

cells is enough to consume most of the oxygen delivered by nearby blood vessels.[70] Notably, HSCs seem to localize at the endosteal surface of bone, which is considered to be highly hypoxic (Figure 3).[71] A role of hypoxia and the HIF pathway in the regulation of HSCs and haematopoiesis is becoming increasingly evident. For example, HSCs are capable of surviving on glycolysis (in a manner dependent on HIF-1α activity) instead of mitochondrial respiration.[72] Moreover, HSCs seem to maintain cell cycle quiescence, and persist in adequate numbers, through tight control of HIF-1α levels; induction of conditional loss of HIF-1α in mice (Mx1-Cre driven) resulted in loss of HSC quiescence and decreased HSC numbers, particularly in conditions of increased stress, whereas the corresponding knockout of VHL protein (which results in supranormal levels of HIF-1α) induced HSC quiescence, but it also impaired the engraftment of transplanted cells.[73]

VEGF also has a survival-promoting function in HSCs, acting through an internal, but not yet fully elucidated, autocrine loop that does not require extracellular binding of VEGF to its cell surface receptor.[74] Moreover, impairment of the HIF-dependent expression of VEGF in HSCs alters the function of these cells *in vivo*, as demonstrated by their impaired regenerative capacity in transplantation assays.[75]

Despite this evidence, the precise mechanisms of how the HIF signalling pathway and/or VEGF regulate the microenvironments inhabited by HSCs or marrow osteoblasts are yet to be determined. It is tempting to speculate that both HIFs and VEGF could affect the survival and/or differentiation of HSCs by modifying the osteoblast and/or the vascular niches.

[H1] Bone regeneration and disease

The HIF signalling pathway and VEGF have been implicated in a number of pathological conditions, including osteoarthritis, and in regenerative processes, such as fracture repair.

[H2] Fracture repair

Successful fracture repair largely recapitulates the various stages of bone development; the appropriate development of blood vessels is, therefore, essential. Bone repair can occur through the two major processes of bone development: ntramembranous ossification, which occurs when the bone segments are stabilized or during distraction osteogenesis and is characterized by direct differentiation of mesebchymal cells into osteoblasts,, or endochondral ossification (for mechanically unstable fractures). Several angiogenic factors, including VEGF, are upregulated during fracture healing.[76] In mice, treatment with a soluble VEGF receptor (VEGFR) or VEGF antagonist resulted in impaired bone formation, whereas local VEGF administration successfully increased both types of bone healing.[77] VEGFR-1 and VEGFR-2 are critically involved in angiogenesis as well as bone formation during fracture repair.[78] During angiogenesis, VEGF stimulates tip cell induction and filopodia formation via VEGFR-2, whereas VEGFR-1 is predominantly expressed in stalk cells and is involved in guiding and limiting tip cell formation.[79] However, VEGFR-1 is not only expressed by endothelial cells but also by monocytes, macrophages, mesenchymal progenitor cells, osteoblasts and osteoclasts, which all contribute to the process of fracture healing.[80] The role of VEGFR-1 in fracture healing is emphasized by the fact that mice lacking the VEGF homolog placental growth factor (PlGF, a pleiotropic cytokine that exerts its effects solely through VEGFR-1), showed impaired bone repair (Figure 4).[81] Several cell types in the bone and marrow

environment, including osteogenic cells, macrophages, and (pre)osteoclasts, can secrete PlGF. [82] [83] Genetic studies revealed that endogenous PlGF is not necessary for vascular development and physiological vessel maintenance in healthy adults.[84] However, in various pathological conditions such as cancer PlGF is greatly upregulated by various stimuli, including hypoxia, and contributes to angiogenesis and to attract inflammatory cells, both critical events in fracture healing.[85,82] PlGF-knockout mice show impaired fracture healing caused by decreased inflammation, osteogenic response and callus remodelling (Figure 4).[81]

From a therapeutic perspective, interfering with the HIF signalling cascade might offer a physiological strategy for improving fracture healing. Genetic activation of the HIF-1α pathway in mature osteoblasts improved bone formation and angiogenesis in a distraction osteogenesis model; the reverse was observed in animals whose osteoblasts lacked HIF-1α.[86] Small molecule inhibitors of the prolyl hydroxylases that normally target HIF-1α for destruction can be used to block HIF-1α degradation, which activates the HIF pathway and in turn increases VEGF production.[86,87] In general, these inhibitors interfere with the co-factors required by these prolyl hydroxylases (either iron chelators or 2-oxyglutarate analogues). Inhibition of the prolyl hydroxylases resulted in increased vascularity and accelerated bone healing in endochondral as well as intramembranous fracture repair,[86,87] suggesting that this approach can be used widely in the clinical management of skeletal repair.

[H2] Osteoarthritis

In articular cartilage from osteoarthritic joints, the expression of VEGF, HIF-1α and HIF-2α is increased.[88] Intra-articular transplantation of genetically modified, muscle-

derived stem cells that express both bone morphogenetic protein 4 (which induces cartilage and bone formation) and soluble VEGFR-1 improved cartilage repair in a rat model of osteoarthritis.[89] Evidence from transgenic animal studies in support of a role for HIF-1α in osteoarthritis is still lacking, but HIF-2α aploinsufficiency prevents cartilage degradation and osteophyte formation in mice.[21] Furthermore, overexpression of HIF-2α achieved by transgenic overexpression is sufficient to trigger cartilage destruction in this model (Figure 5). [90] Notably, the increase in HIF-2α activity in articular cartilage appears to rely on NF-κB signalling rather than regulation by prolyl hydroxylase activity.[21,90] Therefore, a likely sequence of events is that mechanical stress and/or the presence of proinflammatory cytokines induce HIF-2α expression in articular chondrocytes, which promotes the expression of proteins involved in chondrocyte hypertrophy as well as multiple proteases that degrade the cartilaginous extracellular matrix (Figure 5). It is an open question whether changes in oxygen tension could also play a role. All in all, these findings could have important therapeutic implications for this common form of arthritis, as they indicate that inhibition of the HIF-2α signalling pathway could be beneficial for the treatment of osteoarthritis.

[H2] Metastasis to bone

Bone is a common site for the settlement and growth of metastasizing cells, especially of breast and prostate cancers.[91] Tumour cells that engraft in the bone can remain clinically dormant for several years. In response to still unknown triggers, however, these latent tumour cells can achieve full metastatic competence and stimulate osteoclast activity. During resorption of the bone matrix, embedded growth factors are released and these, in turn, promote tumour growth.[91] Micrometastases require adequate vascularization to

grow,[92] which is achieved in metastatic bone lesions through the expression of VEGF.[93] Indeed, blockade of VEGF with bevacizumab in a rat model of metastatic breast cancer reduced the size of osteolytic lesions and inhibited further tumour growth, presumably by inhibiting angiogenesis.[93] Furthermore, PlGF is secreted by osteogenic cells; blocking bone-derived PlGF activity in mice reduced size and number of osteolytic bone metastases by decreasing engraftment of tumour cells in the bone and inhibiting the activation of osteoclasts by tumour cells (Figure 6).[83]

The hypoxic bone microenvironment might also favour metastatic tumour growth. Overexpression of HIF-1α in breast tumour cells promoted the progression of osteolytic bone metastases,[94] whereas the reverse effect was observed with HIF-1α knockdown.[95] Targeting angiogenic factors, such as VEGF and PlGF, or the HIF signalling cascade might, therefore, provide an effective strategy for the treatment of bone metastases (Figure 6), although appropriate preclinical testing is warranted given the modest results of antiangiogenic therapy in patients with cancer.[96]

In a related arena, the HIF signalling pathway is potentially involved in the initiation and progression of human chondrosarcomas.[97–98] Mutations that affect isocitrate dehydrogenases 1 and 2 occur in human chondrosarcomas,[97–98] and lead to depletion of the co-factor α-ketoglutarate—which, in turn, could lead to increased accumulation of HIFs through inhibition of HIF prolyl hydroxylases.[99] The HIF signalling pathway might, therefore, be altered in chrondrosarcomas, which could contribute, at least in part, to the initiation and/or progression of these malignancies.

[H1] Conclusions

Mouse models in which the HIF signalling pathway has been genetically manipulated in the limb bud mesenchyme, chondrocytes, osteoblasts and hematopoietic bone marrow have indicated a critical role for this pathway in bone development, regeneration and disease. Regulation of angiogenesis by VEGF, a key downstream target of HIFs, has also been critically implicated in each of these processes. Identification of the molecular mechanisms that govern the complex mosaic of biological effects of the HIFs needs to be pursued further. In particular, an interesting and informative avenue of research will be investigations of the role of glycolysis and mitochondria in the HIF signalling pathway and its control of cell survival and differentiation.

Review criteria

We searched the PubMed database, using the following search terms (alone or in combination): “cartilage”, “bone”, “hematopoietic marrow”, “HIF”, “VHL”, “VEGF”, “PlGF”, “hypoxia”, “chondrocyte”, “osteoblast”, “osteoclast”, “bone development”, “fracture”, “osteoarthritis”, “bone metastasis” and “chondrosarcomas”. Results were not limited by date of publication, but only full-text papers published in English were selected.

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Author contributions

All authors contributed to researching data for the article, writing the manuscript, and provided substantial contributions to discussions of the content and review or editing of the manuscript before acceptance.

Figure 1. The HIF-1α pathway. In normoxia, the cellular oxygen sensors PHDs hydroxylate specific proline residues (Pro402 and Pro564) of HIF-1α, leading to its proteosomal degradation mediated by pVHL, an E3 ubiquitin ligase. During hypoxia, HIF-1α is not ubiquitinylated or degraded, and acts as a transcription factor that binds to HREs to induce expression of a plethora of target genes, including *VEGF*, which promotes angiogenesis, and genes involved in anaerobic metabolism. Abbreviations: HIF, hypoxia-inducible transcription factor; PHD, prolyl hydroxylases (Egl nine homologs 1 and 2); pVHL, von Hippel–Lindau protein; HRE, hypoxia response element.

Figure 2. The roles of HIF-1α and VEGF in regulating the oxygenation of cartilage during embryonic development. Being an avascular tissue, the central portion of the foetal growth plate becomes hypoxic as it grows (blue). Hypoxia, partly via HIF-1α, induces expression of VEGF by chondrocytes within the growth plate, which stimulates angiogenesis and increases oxygen supply to the chondrocytes. HIF-1α also exerts VEGF-independent functions that regulate the survival of hypoxic chondrocytes, including activating the glycolytic metabolic pathway. Both HIF-1α and VEGF have multiple effects at different stages of endochondral bone development, although the

molecular mechanisms responsible for these pleiotropic functions are still largely unknown. Abbreviations: HIF-1α, hypoxia-inducible transcription factor 1α; VEGF, vascular endothelial growth factor-A.

Figure 3. The HIF and VEGF signalling pathway in bone. The oxygen gradient in the bone marrow renders the osteoblastic HSC niche at the endosteal surface hypoxic. Osteoblasts express HIFs, which modulate bone development and homeostasis and angiogenesis. Some of the effects of HIFs on bone and angiogenesis are mediated by VEGF.. Abbreviations: HSC, hematopoietic stem cell; HIF, hypoxia-inducible transcription factor; VEGFR, VEGF receptor.

Figure 4. Role of the HIF–VEGF–PlGF pathway in fracture repair. As a consequence of fracture blood vessels in the bone rupture, causing the fracture site to become hypoxic. VEGF and PlGF expression stimulates the angiogenic response. Together with other cytokines, PlGF recruits inflammatory cells to clear cellular debris, and induces the proliferation of periosteal cells (a source of osteogenic cells). In the subsequent phases of soft and hard callus formation, PlGF contributes to the turnover of the cartilage matrix and remodelling of the newly formed woven bone by controlling osteoclast formation. Abbreviations: HIF, hypoxia-inducible transcription factor; PlGF, placental growth factor.

Figure 5. Role of the HIF pathway in osteoarthritis. Mechanical stress and/or proinflammatory cytokines activate NF-κB signalling that controls HIF-2α expression in

articular chondrocytes. HIF-2α promotes the expression of proteins involved in chondrocyte hypertrophy (IHH and collagen type X) as well as MMP13, which degrades the cartilage extracellular matrix, and VEGF. The result is thinning of the articular cartilage at the centre of the joint and osteophyte formation at the periphery. Abbreviations: HIF, hypoxia-inducible transcription factor; IHH, Indian hedgehog homolog; MMP13, matrix metalloproteinase-13.

Figure 6. Role of the HIF–VEGF–PIGF pathway in tumour metastasis to bone. PlGF secreted by osteogenic cells favours tumour cell engraftment in the bone microenvironment. Surviving tumour cells form micrometastases and induce PlGF secretion in osteogenic cells. Increased PlGF levels advance the switch to the osteolytic phase by stimulating osteoclastogenesis, which controls the formation of macrometastases. HIF and VEGF also contribute to the angiogenic switch during metastatic growth. Abbreviations: HIF, hypoxia-inducible transcription factor; PlGF, placental growth factor.

BIOGRAPHIES

Christa Maes graduated in bioengineering from KU Leuven, Belgium. She obtained her PhD in angiogenic signalling molecules in bone development and repair in 2004, and conducted postdoctoral studies in osteoblast and chondrocyte biology at Harvard Medical School, Cambridge, MA, USA. She currently works as an assistant professor at KU Leuven.

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Ernestina Schipani obtained her medical degree and PhD at the University of Pisa, Italy. She is currently a full professor of medicine and of anatomy and cell biology at Indiana University Medical School, Indianapolis, IN, USA. Dr Schipani has a longstanding interest in the study of cartilage and bone biology, and has authored more than 100 publications.

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