

# Hypoxic regulation of osteoclast differentiation and bone resorption activity

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**Abstract:** Bone integrity is maintained throughout life via the homeostatic actions of bone cells, namely, osteoclasts, which resorb bone, and osteoblasts, which produce bone. Disruption of this balance in favor of osteoclast activation results in pathological bone loss, which occurs in conditions including osteoporosis, rheumatoid arthritis, primary bone cancer, and cancer metastasis to bone. Hypoxia also plays a major role in these conditions, where it is associated with disease progression and poor prognosis. In recent years, considerable interest has arisen in the mechanisms whereby hypoxia and the hypoxia-inducible transcription factors, HIF-1 $\alpha$  and HIF-2 $\alpha$ , affect bone remodeling and bone pathologies. This review summarizes the current evidence for hypoxia-mediated regulation of osteoclast differentiation and bone resorption activity. Role(s) of HIF and HIF target genes in the formation of multinucleated osteoclasts from cells of the monocyte–macrophage lineage and in the activation of bone resorption by mature osteoclasts will be discussed. Specific attention will be paid to hypoxic metabolism and generation of ATP by osteoclasts. Hypoxia-driven increases in both glycolytic flux and mitochondrial metabolic activity, along with consequent generation of mitochondrial reactive oxygen species, have been found to be essential for osteoclast formation and resorption activity. Finally, evidence for the use of HIF inhibitors as potential therapeutic agents targeting bone resorption in osteolytic disease will be discussed.

**Keywords:** ATP, hypoxia-inducible factor, HIF, osteolysis, glycolysis, reactive oxygen species, mitochondrial metabolism

## Introduction

Hypoxia is a characteristic microenvironmental component of numerous pathological conditions, where it correlates with disease progression and/or severity. Many of these conditions are also associated with bone loss, including cancer,<sup>1,2</sup> rheumatoid arthritis,<sup>3,4</sup> osteoporosis,<sup>5</sup> and bone fracture,<sup>6</sup> as well as extra-skeletal conditions such as obstructive pulmonary disease.<sup>7</sup>

Bone remodeling during development and bone integrity throughout life are normally regulated by a balance between bone formation, performed by osteoblasts, and bone resorption, performed by osteoclasts. Pathological bone loss occurs when this homeostatic relationship is disturbed. Overactivation of osteoclasts is directly responsible for the resorptive bone loss evident in rheumatoid arthritis,<sup>8,9</sup> osteoporosis,<sup>10</sup> and cancer metastasis to bone.<sup>11</sup>

Given that hypoxia and hypoxia-inducible factor (HIF) are also present in these conditions, it is important to understand how hypoxia pathways affect the differentiation and activity of osteoclasts. This review summarizes the research into effects of

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hypoxia and HIF on bone-resorbing osteoclasts, highlighting potential new targets for the development of antiresorptive therapies.

## Hypoxia stimulates osteoclastogenesis in vitro

Osteoclasts form by the fusion of CD14<sup>+</sup> monocyte or macrophage precursors, in the presence of macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL), to produce mature multinucleated cells.<sup>12,13</sup> The mature osteoclasts then attach to mineralized bone and acidify the extracellular environment at the cell-bone interface, releasing bone minerals and exposing the organic matrix to resorption by the secreted acid protease cathepsin K.<sup>14,15</sup> Cells of the monocyte–macrophage lineage from which osteoclasts derive have long been known to be activated by hypoxia,<sup>16,17</sup> suggesting that monocyte–osteoclast differentiation and osteoclast activity might also be increased at low O<sub>2</sub> tensions.

The first study into effects of hypoxia on osteoclast formation used bone marrow cells flushed from mouse long bones as a source of monocytic precursors. When differentiated in vitro in the presence of M-CSF and RANKL, maximal hypoxic stimulation was achieved at 2% O<sub>2</sub>, which produced a fourfold increase in osteoclast number and a threefold increase in resorption activity per osteoclast at the end of the differentiation period.<sup>18</sup> Similar results were found using circulating monocytes from feline<sup>19</sup> and human<sup>20</sup> peripheral blood mononuclear cells (PBMCs) as osteoclast precursors. Attention to the hypoxia protocol in these studies reveals that the cells were actually exposed to a hypoxia/re-oxygenation schedule, rather than hypoxia per se, during the period of differentiation. The need for re-oxygenation during hypoxic differentiation was confirmed in a separate study; steady-state exposure to 2% O<sub>2</sub> in a gloved hypoxic workstation dramatically inhibited osteoclast formation and resorption due to extensive cell death.<sup>21</sup>

Acute exposure to hypoxia also increases the ability of mature osteoclasts to resorb bone. Resorption is normally measured in vitro by culturing osteoclasts on dentine (elephant ivory) discs and either quantifying the area of resorption tracks or assaying release of cross-linked C-telopeptide of type I collagen (CTXI) into the supernatant. Osteoclasts derived from feline<sup>19</sup> or human<sup>21–23</sup> PBMC, as well as mature human osteoclasts curetted from the primary bone tumor giant cell tumor of bone,<sup>21</sup> exhibited a twofold to fourfold increase in resorption after 24 hours exposure to 2% O<sub>2</sub>.

No effect was observed in primary osteoclasts from disaggregated rat bones.<sup>18</sup>

The fact that hypoxia can stimulate the formation and activity of human osteoclasts derived from PBMCs, where there is effectively no stromal cell support,<sup>20,21</sup> as well as from pure populations of CD14<sup>+</sup> monocytes,<sup>22,23</sup> suggests that the osteoclastogenic response to hypoxia is an intrinsic property of this cell lineage.

## Hypoxia stimulates osteoblast-mediated osteoclastogenesis

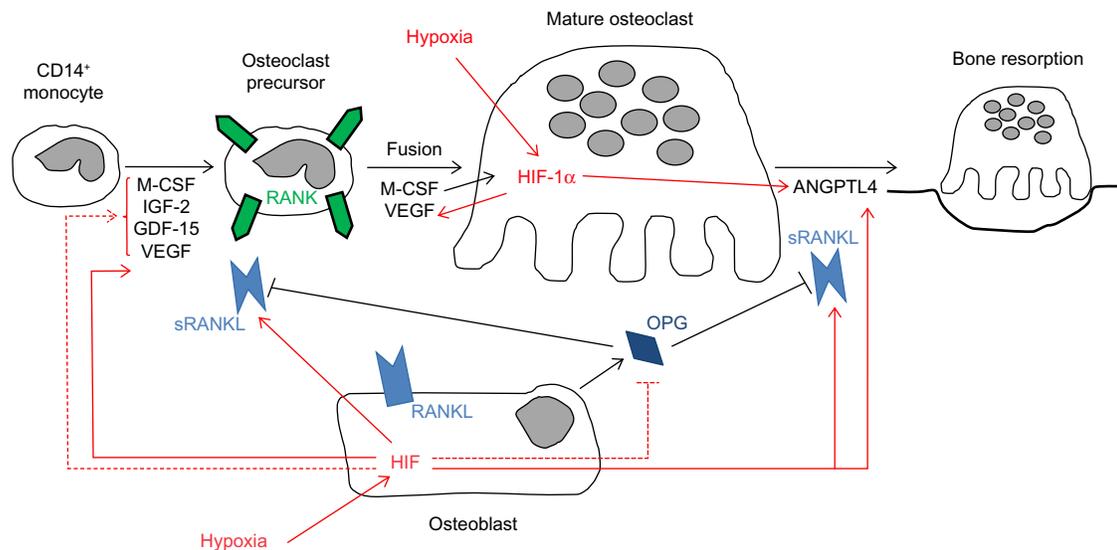
Obviously, in the in vivo situation, monocytes and osteoclasts do not exist in isolation but are surrounded by osteoblasts, fibroblasts, and other cellular components of the bone microenvironment that will also be exposed to local hypoxia. Co-culture of monocytes with stromal cells including osteoblasts, fibroblasts, and cancer cells has revealed that hypoxia stimulates local production of pro-osteoclastogenic cytokines including RANKL,<sup>24,25</sup> vascular endothelial growth factor (VEGF),<sup>24–27</sup> M-CSF,<sup>27</sup> insulin-like growth factor 2,<sup>28</sup> and growth differentiation factor 15,<sup>29</sup> as well as inhibiting production of osteoprotegerin (OPG), a soluble decoy receptor for RANKL that inhibits osteoclast formation and activity.<sup>30</sup>

Discussion of the regulation and mechanisms of action of the full range of osteoclastogenic cytokines produced by support cells within the hypoxic bone microenvironment is beyond the scope of this review. However, it is clear that hypoxia, both directly and indirectly, has a large impact on osteoclast formation and bone resorption activity (Figure 1). This is supported by a study in mice lacking the Fos-related protein Fra-2, which form giant osteoclasts due to the presence of hypoxia in their long bones.<sup>31</sup> Conversely, exposure to hyperbaric oxygen reduces human osteoclast formation and bone resorption in vitro.<sup>32,33</sup>

## Role of HIF in osteoclast-mediated bone resorption

Expression of HIF-1 $\alpha$  and HIF-2 $\alpha$  protein by human osteoclasts was first described as recently as 2008.<sup>26</sup> Mature human monocyte-derived osteoclasts in vitro have since been shown to stabilize HIF-1 $\alpha$  and HIF-2 $\alpha$  in response to either hypoxia or hypoxia mimetics such as MG132, CoCl<sub>2</sub>, desferrioxamine, or dimethylxalylglycine.<sup>20–23,26</sup> HIF expression also increases on exposure to osteoclastogenic cytokines<sup>26</sup> and, therefore, during monocyte–osteoclast differentiation.<sup>5,20</sup>

However, it seems likely that expression of HIF alone is insufficient to stimulate cell autonomous osteoclastogenesis.



**Figure 1** Cytokine-mediated effects of hypoxia on osteoclast differentiation and bone resorption activity.

**Notes:** Hypoxic stimulation of cytokine secretion by osteoclasts and osteoblasts is shown as either HIF-dependent (solid red lines) where known or as HIF-independent/unknown (dashed red lines). The majority of osteoclastogenic cytokines affect monocyte–osteoclast differentiation. Relatively few are known to specifically affect bone resorption activity (RANKL, ANGPTL4).

**Abbreviations:** M-CSF, macrophage colony stimulating factor; IGF-2, insulin-like growth factor 2; GDF-15, growth differentiation factor 15; VEGF, vascular endothelial growth factor; RANK, receptor activator of nuclear factor kappa B; RANKL, receptor activator of nuclear factor kappa B ligand; OPG, osteoprotegerin; HIF-1 $\alpha$ , hypoxia-inducible factor 1-alpha; ANGPTL4, angiopoietin-like 4; sRANKL, soluble RANKL.

Formation of murine osteoclasts was shown to be inhibited in the presence of dimethylxalylglycine or desferrioxamine, or when monocytes were transfected with a constitutively active form of HIF-1 $\alpha$ .<sup>34</sup> Similarly, differentiation of human PBMC was inhibited in the presence of CoCl<sub>2</sub>.<sup>35</sup> This is in agreement with our unpublished observations that CoCl<sub>2</sub> and desferrioxamine inhibit human monocyte–osteoclast differentiation. However, other groups have reported that cobalt stimulates differentiation of murine osteoclasts.<sup>36</sup> A specific role for HIF in the process of monocyte–osteoclast differentiation therefore remains to be defined.

On the other hand, HIF does appear to be responsible for the hypoxia-induced increase in bone resorption by mature osteoclasts. HIF-1 $\alpha$  siRNA completely ablated the hypoxic increase in resorption by human monocyte-derived osteoclasts, although HIF-2 $\alpha$  siRNA had no effect.<sup>21,22</sup> Hypoxic osteoclasts show increased secretion of the HIF-regulated osteoclastogenic cytokine VEGF<sup>20,21</sup> and resorption-promoting angiopoietin-like 4 (ANGPTL4; Figure 1).<sup>22</sup> These were not apparently directly responsible for the HIF-1 $\alpha$ -dependent increase in osteoclast activity, however,<sup>21,22</sup> suggesting that HIF plays other role(s) to increase osteoclast activity.

## HIF and osteoclast metabolism

The bone resorption process involves osteoclasts binding to bone via  $\alpha v \beta 3$  integrin and then forming an F-actin-rich

seal to isolate a resorptive compartment. Active transport of protons across the bone-apposing membrane by vacuolar H<sup>+</sup> ATPase along with Na,K-ATPase, Ca-ATPase, and gastric H,K-ATPase<sup>37</sup> acidifies this compartment, releasing bone minerals and exposing the organic matrix. The matrix is subsequently resorbed by the secreted acid protease cathepsin K.<sup>14</sup> Osteoclasts are also highly motile, all of which accumulates to make osteoclast-mediated bone resorption, an energy-intensive process with a high demand for ATP.<sup>15,38</sup> Osteoclasts contain numerous mitochondria,<sup>39</sup> associated with high expression of tricarboxylic acid cycle and oxidative phosphorylation enzymes,<sup>40</sup> and exhibit high rates of oxygen consumption.<sup>41</sup> This implies that high mitochondrial metabolic activity drives ATP production in these cells.

It is therefore interesting to consider how osteoclasts generate sufficient ATP to support the observed increase in bone resorption under hypoxia. Cellular adaptation to hypoxia generally entails switching to anaerobic metabolism – a HIF-mediated survival mechanism involving inhibition of mitochondrial ATP production in order to prevent accumulation of toxic levels of reactive oxygen species (ROS).<sup>42,43</sup> This is a multistep process. Initially, HIF increases the efficiency of complex IV of the mitochondrial electron transport chain (ETC) with respect to the amounts of ATP and ROS produced, triggering a switch in expression of cytochrome *c* oxidase subunits from COX4-1 to COX4-2.<sup>44</sup> When this is unable to maintain energy/redox homeostasis, a switch

occurs from mitochondrial to purely glycolytic metabolism. HIF stimulates increased expression of glucose transporters and glycolytic enzymes to increase flux through the glycolytic pathway.<sup>45</sup> It also increases expression of pyruvate dehydrogenase (PDH) kinase (PDK), which phosphorylates and inactivates PDH, the mitochondrial enzyme responsible for converting pyruvate into acetyl co-enzyme A.<sup>46,47</sup> This reduces flux through the mitochondrial tricarboxylic acid cycle and ETC and again reduces accumulation of ROS. As a final response, HIF induces expression of BCL2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3), which initiates mitochondrial autophagy and further reduces accumulation of ROS.<sup>48</sup>

## Glycolysis

The monocyte/macrophage population from which osteoclasts derive, which must also be able to function in hypoxic environments, relies heavily on HIF-1 $\alpha$ -mediated transcription of glycolytic genes to produce ATP.<sup>49</sup> Despite this already high baseline glycolytic activity, the glycolytic rate, measured either as glucose consumption or lactate production, increases further during monocyte-osteoclast differentiation.<sup>41,50</sup>

Glucose is the principal energy source necessary for bone degradation.<sup>50,51</sup> Within the physiological range, an increased glucose concentration rapidly increases the intracellular ATP:ADP ratio.<sup>52</sup> Longer exposure activates transcription of the A-subunit of vacuolar H<sup>+</sup> ATPase,<sup>53</sup> which interacts directly with the glycolytic enzyme phosphofructokinase-1.<sup>54</sup> This interaction is thought to micro-compartmentalize glycolytic ATP generation at the required intracellular location, directly linking glycolysis and osteoclast activation. Indeed, inhibition of glycolysis could be a therapeutic antiresorptive option. Glycolytic inhibitors reduce bone resorption in animal models of disease<sup>55,56</sup> and have been shown to induce clinical remission in rheumatoid arthritis.<sup>57,58</sup>

Hypoxia then elevates the already high basal glycolytic rate of osteoclasts still further. Hypoxic osteoclasts demonstrate increased expression of HIF-regulated glucose transporters (*SLC2A1* mRNA<sup>23,59</sup> and Glut-1 protein<sup>26</sup>) and glycolytic enzymes (*PGK1*, *PFKFB4*, *ALDOC*, and *LDHA*<sup>23</sup>), resulting in a HIF-1 $\alpha$ -dependent increase in glucose consumption.<sup>23</sup> Lactate production also increases, although the ratio of glucose consumption to lactate production remained unchanged, suggestive of a hypoxic increase in flux through the glycolytic pathway but not of a switch to anaerobic glycolysis<sup>23</sup> (Figure 2).

Glucose uptake remains essential for osteoclast activity in hypoxia as depletion of glucose severely reduced the

generation of intracellular ATP by hypoxic osteoclasts.<sup>23</sup> Increased glycolysis by actively resorbing hypoxic osteoclasts may also occur in vivo. Positron emission tomography with 2-(fluorine-18)fluoro-2-deoxy-D-glucose (<sup>18</sup>FDG) in benign primary bone tumors can distinguish those containing many osteoclasts from those where osteoclasts are sparse,<sup>60</sup> additionally correlating with markers of hypoxia.<sup>61</sup>

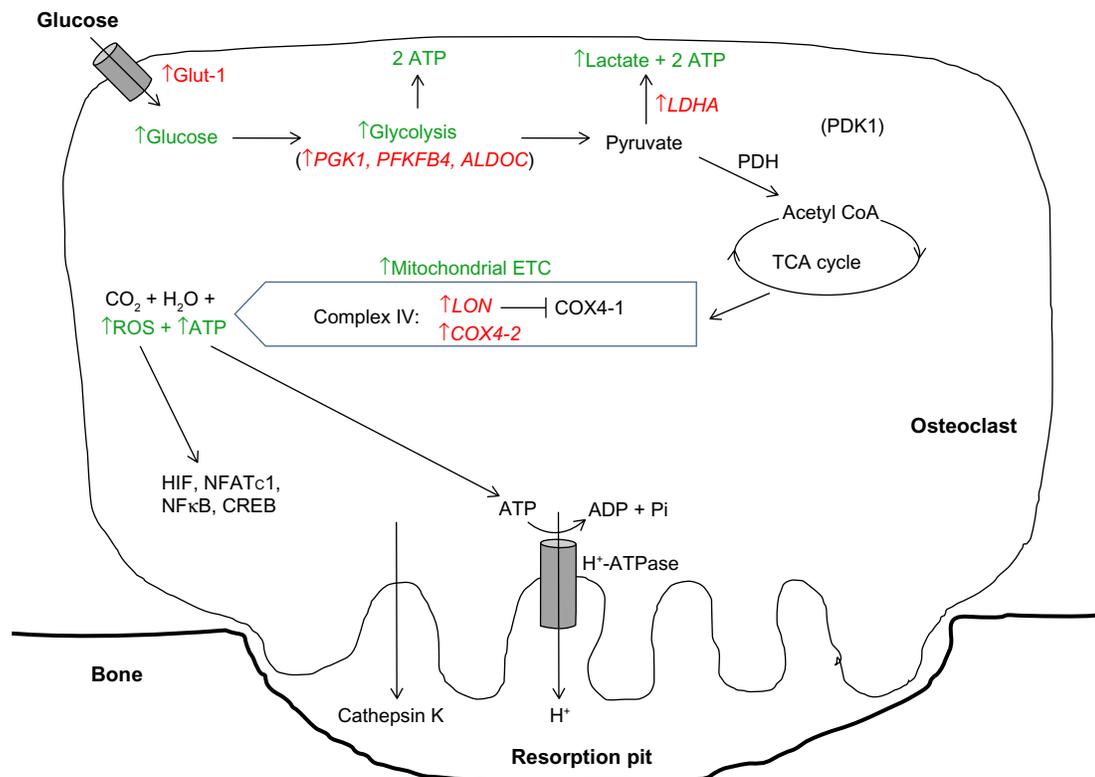
## Mitochondrial metabolism

In line with the generally accepted switch to anaerobic metabolism in hypoxia, most hypoxic cells exhibit reduced concentrations of intracellular ATP and reduced mitochondrial metabolic flux.<sup>46,47</sup> However, elevated concentrations of ATP were observed in hypoxic osteoclasts, as well as increased mitochondrial reductase activity within the ETC. There was also no reduction in O<sub>2</sub> consumption via the ETC under hypoxia; this remained close to maximal and was even more sensitive to ETC inhibition with rotenone than in the corresponding normoxic cells.<sup>23</sup> This hypoxic increase in ETC activity was at least partially dependent on HIF-1 $\alpha$ , mediated by apparently selective utilization of components of the classical HIF-mediated metabolic switch to anaerobic respiration that increase or maintain pathway activity (the COX subunit switch, increased glycolytic rate), while neither inhibiting PDH activity nor stimulating BNIP3 production<sup>23</sup> (Figure 2).

HIF-1 $\alpha$ -mediated induction of PDK1 normally results in phosphorylation of PDH and inhibition of PDH activity. However, in mature human osteoclasts, hypoxia had no effect on either PDK1 expression or PDH activity, nor were these affected by HIF-1 $\alpha$  siRNA.<sup>23</sup> PDH can also be inhibited by hypoxic phosphorylation and activation of AMP-activated protein kinase (AMPK), via induction of expression of PDK4.<sup>62,63</sup> However, in osteoclasts, hypoxia dramatically inhibited AMPK phosphorylation and so inactivated AMPK.<sup>23</sup>

As an alternative to hypoxia, AMPK can be activated by reduced intracellular ratios of ATP:ADP or ATP:AMP, hypoxic activation occurring via a mechanism independent of changes in intracellular energy status.<sup>63,64</sup> It may be that high levels of ATP production in hypoxic osteoclasts increase the intracellular ratio of ATP:AMP and override hypoxic mechanisms of AMPK activation in favor of de-phosphorylation and inactivation. AMPK inhibition would be necessary for hypoxic resorption to occur, as AMPK inhibits osteoclast differentiation and activity.<sup>65</sup>

The classical hypoxic attenuation of PDH activity is therefore prevented in osteoclasts by blockade of at least two pathways that usually contribute to its inhibition, allowing



**Figure 2** Modification of the HIF-mediated switch to anaerobic respiration in osteoclasts.

**Notes:** Osteoclasts specifically increase flux through both the glycolytic pathway and mitochondrial ETC to provide ATP and ROS necessary for bone resorption. HIF-regulated genes are shown in red. Green type indicates downstream effects of modulation of HIF target genes.

**Abbreviations:** Glut-1, glucose transporter 1; ATP, adenosine triphosphate; PGK1, phosphoglycerate kinase 1; PFKFB4, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4; ALDOC, aldolase C; LDHA, lactate dehydrogenase A; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; TCA, tricarboxylic acid; COX4-1/2, cytochrome c oxidase subunit 4 isoform 1/2; ETC, electron transport chain; ROS, reactive oxygen species; HIF, hypoxia-inducible factor; NFκB, nuclear factor kappa B; NFATc1, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1; CREB, cAMP response element-binding protein; LON, lon protease homologue, mitochondrial; ADP, adenosine diphosphate; coA, co-enzyme A.

continued mitochondrial metabolic flux under hypoxia. As would be expected, hypoxic continuation of oxidative phosphorylation leads to accumulation of significant amounts of mitochondrial ROS in hypoxic osteoclasts.<sup>23,66,67</sup>

## Reactive oxygen species

ROS, usually in the form of H<sub>2</sub>O<sub>2</sub> or superoxide, are essential for osteoclast function. During monocyte–osteoclast differentiation, RANKL interacts with RANK on the surface of precursor cells to activate a range of signaling pathways. RANKL signaling involves production of ROS by nicotinamide adenine dinucleotide phosphate oxidase following binding of TNF receptor-associated factor 6 to the cytoplasmic domain of RANK. These ROS then serve as second messengers in the MAPK, NFκB, and Ca<sup>2+</sup> mobilization pathways,<sup>68,69</sup> which combine to promote expression of transcription factors including nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATc1). NFATc1 is essential for osteoclastogenesis, promoting transcription of genes such as tartrate-resistant

acid phosphatase (TRAP), calcitonin receptor, cathepsin K, and pro-fusion genes.<sup>70</sup>

ROS also regulate effects on osteoclast-mediated bone resorption and survival. For example, following degradation of bone matrix in the resorption compartment by cathepsin K, osteoclasts endocytose the initial degradation products, along with the cathepsin K, then transcytose these vesicles through the osteoclast. TRAP-containing vesicles fuse with the transcytotic vesicles and cathepsin K digests TRAP to activate its ROS-generating activity. These ROS complete the degradation of the matrix components during their transcytosis.<sup>71</sup> ROS-induced survival mechanisms are initiated by association of c-Src with Src homology 2 domain-containing phosphatase 1. This association activates c-Src, resulting in degradation of the pro-apoptotic protein Bim.<sup>72</sup>

However, hypoxia has been shown to specifically result in accumulation of mitochondrial ROS in osteoclasts.<sup>23,66,67</sup> Mitochondrial ROS are essential for hypoxic enhancement of osteoclast differentiation and resorption, which is reversed by the mitochondria-specific antioxidant MitoQ. MitoQ also

prevents hypoxic induction of NF $\kappa$ B, the calcineurin-NFAT pathway, adenosine 3',5'-cyclic adenosine monophosphate response element-binding protein and, interestingly, HIF – all key signaling molecules in osteoclast differentiation and activity.<sup>67,73</sup> This mitochondrial ROS pathway is proposed to be mediated by mitochondrial respiratory stress signaling, induced by hypoxic release of Ca<sup>2+</sup> from the endoplasmic reticulum, which stimulates production of mitochondrial ROS.<sup>74</sup> Calcium uptake by mitochondria is another mechanism that could contribute to the observed hypoxic increase in oxidative phosphorylation, having been shown to activate isocitrate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, and PDH activity.<sup>75</sup>

The importance of mitochondrial ROS in osteoclastogenesis has recently been demonstrated in mice overexpressing mitochondria-targeted catalase specifically in osteoclasts. These mice had increased bone mass due to a reduction in osteoclast formation and survival and were also protected from ovariectomy-induced bone loss.<sup>76</sup> This demonstrates the importance of mitochondrial ROS to osteoclast activation and provides support for the hypothesis that accumulation of ROS under hypoxia directly promotes bone resorption.

## HIF and osteoclast survival

Despite being resistant to short-term exposure to high levels of ROS, osteoclasts are relatively sensitive to hypoxia when compared with other cell types. At the 2% O<sub>2</sub> optimal for stimulating osteoclast activity, osteoclast numbers fell by 16% and 35% after 24 hours and 72 hours of exposure, respectively.<sup>21,23</sup> After 24-hour exposure to 2% O<sub>2</sub>, one in five remaining osteoclasts exhibited compromised membrane integrity, as assessed by trypan blue uptake through the aqueous pores associated with membrane damage.<sup>21</sup>

Strikingly, re-oxygenation reversed the hypoxic increase in membrane permeability and rescued osteoclasts from the early stages of cell death.<sup>21</sup> Reversible plasma membrane permeability does occur in other cell types,<sup>77,78</sup> and other features of early cell death, including plasma membrane phosphatidylserine exposure<sup>79–81</sup> and mitochondrial swelling,<sup>78,82</sup> are also reversible. This recovery explains how hypoxia/re-oxygenation is able to stimulate osteoclastogenesis,<sup>18–21</sup> whereas continuous hypoxic exposure is inhibitory.<sup>21</sup> Hypoxia/re-oxygenation occurs during injury, ischemia, and reperfusion and is the more likely microenvironmental characteristic to be experienced by monocytes and osteoclasts during *in vivo* differentiation than static hypoxia.

Sensitivity to hypoxia-induced cell death is likely to be the consequence of maintaining high rates of oxidative

phosphorylation in a hypoxic environment; eventually, ROS accumulation would be expected to exceed the osteoclasts' anti-apoptotic capacity. HIF-1 $\alpha$  appears to regulate both aspects of this phenomenon. HIF-1 $\alpha$  siRNA has been shown to prevent hypoxic induction of bone resorption, block the hypoxic increase in glucose consumption, and reduce hypoxic mitochondrial ETC activity, but it also rescues osteoclasts from cell death induced by chronic hypoxic exposure.<sup>21,23</sup>

This led to the hypothesis that, in hypoxic osteoclasts, functional HIF-1 $\alpha$ -dependent pathways initially increase ATP production and bone resorption, but lack of activation of HIF-1 $\alpha$ -dependent survival pathways eventually results in cell death. As osteoclasts are anyway short-lived cells that cannot be allowed to resorb indefinitely, permitting progressive accumulation of ROS under hypoxia may be an adaptive mechanism enabling rapid bone resorption in the short term, while ensuring that the process is halted in the absence of re-oxygenation.

## In vivo effects of HIF inhibition

HIF expression by osteoclasts *in vivo* has only been recently described in ovariectomized (OVX) mice which develop osteoporosis,<sup>5</sup> in the multinucleated giant cells associated with giant cell tumor of bone,<sup>26</sup> and in resorbing osteoclasts within the rheumatoid synovium.<sup>59</sup> However, given that hypoxia and HIF are generally associated with disease progression, HIF inhibition<sup>83</sup> had already been approached as a potential method of improving disease severity in a number of bone resorption conditions.

In murine models of osteolytic breast cancer, treatment with 2-methoxyestradiol (2ME) and its analogs induced apoptosis of osteoclasts and their precursors, protected against tumor-induced osteolysis *in vivo* and inhibited bone resorption *in vitro*.<sup>84,85</sup> Similarly, in murine models of rheumatoid arthritis treatment with endostatin, bortezomib or 2ME improved incidence and severity of arthritis, as well as scores for subchondral bone erosion.<sup>86–89</sup> In OVX mice, 2ME or apigenin preserved bone mineral density, improved concentrations of serum markers of bone turnover, and inhibited osteoclast formation *ex vivo*.<sup>5,87,90</sup>

Some of this inhibitory effect is likely via direct actions of HIF inhibition on osteoclast activity. Ovariectomy results in estrogen depletion, which has been shown to stabilize HIF-1 $\alpha$  protein in osteoporosis-associated osteoclasts in a manner independent of the oxygenation status.<sup>5</sup> Using osteoclast-specific conditional HIF-1 $\alpha$  knockout mice, it was shown that HIF-1 $\alpha$  was essential for the bone loss seen under conditions of estrogen depletion, due to reduced rates of osteoclast formation and reduced bone resorption.<sup>5</sup>

Another contributory factor is likely to be inhibition of secretion of HIF-regulated osteoclastogenic factors. VEGF and ANGPTL4 stimulate osteoclast differentiation<sup>91</sup> and resorption activity,<sup>22</sup> respectively. Serum concentrations of both factors are elevated in patients with rheumatoid arthritis, with high serum concentrations being associated with elevated markers of bone resorption.<sup>59,92</sup>

## What about the osteoblasts?

This review has focused on HIF-mediated regulation of osteoclast activity, the data for which indicates HIF inhibition as a good strategy for targeted therapies to prevent or inhibit pathological bone loss in a number of different conditions.

However, as was mentioned in the “Introduction” section, bone remodeling is regulated by a balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption. The processes of bone formation (osteogenesis) and angiogenesis are tightly linked during bone development and bone repair, and HIF has been shown to regulate this osteogenic–angiogenic coupling. Using mice with osteoblast-specific deletions in either the von Hippel-Lindau gene (*VHL*) or *HIF-1 $\alpha$* , it was shown that hypoxia promotes bone formation by osteoblasts in vivo via HIF-1 $\alpha$ -mediated induction of pro-angiogenic cytokines such as VEGF.<sup>93</sup> Subsequent work confirmed that HIF plays a central role in regulating bone formation during skeletal development.<sup>94,95</sup>

This, and other, osteoblast-directed research led to the hypothesis that HIF pathway activation might be a therapeutic strategy to ameliorate pathological bone loss. Treatment with either HIF prolyl hydroxylase domain enzyme inhibitors or CoCl<sub>2</sub> has been described to improve bone health in murine models of fracture<sup>96–98</sup> and osteoporosis<sup>99,100</sup> by stimulating angiogenesis and concomitant bone formation.

To delve into the reasons behind the apparent discrepancy in these approaches is beyond the remit of this review. Taken at face value, it would seem that osteoclast activity is more sensitive to HIF inhibition and that bone formation is more sensitive to HIF activation, potentially due to HIF-dependent induction of OPG, an inhibitor of osteoclast activity.<sup>95,101</sup> Intriguingly, intervention in either direction apparently tips the homeostatic balance back in favor of maintaining or improving bone integrity.

## Conclusion

HIF, specifically HIF-1 $\alpha$ , is closely involved in both monocyte–osteoclast differentiation and bone resorption by mature osteoclasts, especially within the hypoxic microenvironment so closely associated with many pathological bone resorption

conditions. HIF stimulates the expression of cytokines that regulate the differentiation and resorption process. It also increases both the glycolytic and mitochondrial metabolic rate in order to generate sufficient ATP to support the hypoxic increase in bone resorption. Generation of mitochondrial ROS, a by-product of maintaining mitochondrial respiratory activity under hypoxic conditions, is itself necessary to enable this high rate of resorption. These data suggest HIF as an attractive therapeutic target in osteolytic disease, although further research is urgently needed in order to align this with work on osteoblastic aspects of bone disease.

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## Disclosure

The author reports no conflicts of interest in this work.

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