

Acroosteolysis in systemic sclerosis: An insight into hypoxia-related pathogenesis (Review)

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Abstract. Acro-osteolysis, or bony resorption of the terminal digital tufts, is a well-recognized, but under-researched, feature of systemic sclerosis. The mechanisms that disturb local homeostatic balance of bone formation and resorption in favor of osteoclast activation and pathological bone loss remain to be established. Vascular alterations and reduced capillary density impair tissue oxygenation in systemic sclerosis, and the resulting hypoxia might contribute directly to the disease progression. In this paper we summarize the current evidence for hypoxia as the common pathophysiological denominator of digital vasculopathy and enhanced osteoclastic activity in systemic sclerosis-associated acroosteolysis. The hypoxia-inducible transcription factor HIF-1 α and VEGF signaling has a critical role in regulating osteoclastic bone-resorption and angiogenesis, and increased osteoclastogenesis and higher VEGF levels may contribute to acroosteolysis in systemic sclerosis. The cells of the osteoblast lineage also have important roles in angiogenic-osteogenic coupling. The research in this field might help limiting the disability associated with the disease.

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Abbreviations: AO, acroosteolysis; Ang, angiopoietin; ANGPTL, angiopoietin-like protein; BMP, bone morphogenetic protein; CTGF, connective tissue growth factor; HIF- α , hypoxia-inducible factor α ; NVC, nailfold videocapillaroscopy; SSc, systemic sclerosis; VEGF, vascular endothelial growth factor

Key words: acroosteolysis, systemic sclerosis, hypoxia, angiogenesis, VEGF, bone cells

1. Introduction

Systemic sclerosis (SSc) is an autoimmune disease characterized by microvascular impairment, immune dysregulation and fibrosis (1). Acroosteolysis (AO), a distinct pattern of bone resorption affecting the distal portion of the fingers, which occurs in 6-65% of patients with SSc, is an incompletely addressed feature of the disease that contributes to disability and pain (2-6).

AO is very suggestive of SSc, although not pathognomonic, being encountered in several congenital or acquired entities (7). The clinical and radiographic course of SSc-related AO is variable, as stabilization of osteolysis can occur in some patients, while in others, osteolysis progresses towards complete loss of distal phalanges and even to digital telescoping. Osteolysis generally starts on the palmar aspect of the tuft, leading to sharpening of the distal phalanx and, in rare cases, the middle phalanx (5). Ultrasonography is similar to radiography in the detection of AO, revealing interruption of the cortical contours and increased vascularization at bone resorption sites (8). Rarely resorption may affect other bones, such as the mandible, distal radius and ulna, distal clavicle, ribs or even cervical vertebrae (9). It is not clear whether AO occurs preferentially within the limited form of disease (4). However, AO has been suggested to be associated with the duration of disease, digital ulcers, severe digital ischemia, severe Raynaud's phenomenon, late capillaroscopic pattern with severe capillary loss, pulmonary arterial hypertension, hand calcinosis, secondary hyperparathyroidism and in general with more severe disease (2,4).

The pathogenesis of AO in SSc is not well understood; presumed mechanisms include a reduction of vascular supply due to digital occlusive vasculopathy and later due to external compression from skin tightening, impaired angiogenesis due to defective sprouting following hand microtrauma, nerve alteration due to compressive neuropathy in the carpal, ulnar or cubital tunnel, and occult hyperparathyroidism resulting from vitamin D deficiency (7-10). However, the main contributors to AO in SSc appear to be vascular impairment, as well as an imbalance in bone remodeling favoring resorption. Bone homeostasis requires an adequate vascular supply, and a close spatial-temporal association exists between angiogenesis and osteogenesis during skeletal development, bone growth and fracture healing (11). Angiogenesis is a crucial component of

bone remodeling. While tissue hypoxia normally stimulates angiogenesis, SSc is characterized by a decreased and inefficient angiogenic response, leading to a failure to replace damaged vessels and a reduction of capillary density (12). The present review addresses the hypothesis that hypoxia and chronic ischemia-derived stimuli are involved in the development and progression of SSc-associated AO.

2. Hypoxia in systemic sclerosis: The HIF pathway and its dysregulation in angiogenesis and osteoclastogenesis

In SSc chronic tissue hypoxia results from obliterative vasculopathy, reduced capillary density and the overproduction of extracellular matrix proteins causing impairment of diffusion from blood vessels to cells (12). The master transcriptional regulator of the adaptive response to hypoxia is hypoxia-inducible factor-1 α (HIF-1 α) (13). HIF-1 α negative regulators are HIF prolyl hydroxylases (PHDs) and Von Hippel-Lindau tumor suppressor protein (14). Hypoxia promotes the increase of HIF-1 α in virtually all cell types; in normoxia, HIF-1 α is rapidly destroyed following hydroxylation by PHDs, whereas during hypoxia, prolyl-hydroxylation is blocked, leading to HIF-1 α stabilization, nuclear translocation and gene activation (13). The HIF pathways regulate pro-angiogenic genes, including vascular endothelial growth factor (VEGF), angiopoietin (Ang)-1 and Ang-2, platelet-derived growth factor (PDGF), basic fibroblast growth factor and monocyte chemoattractant protein-1 (15). Therefore, HIF-1 is a global mediator of the angiogenic response to hypoxia.

Vascular perspective. Vascular homeostasis depends on the balance of at least two main systems: VEGF/VEGF receptor (VEGFR) and the Ang-Tie ligand-receptor system. Numerous other factors are involved, including endostatin, angiostatin, PDGF and fibroblast growth factor-2.

VEGF/VEGF receptor system. VEGF, one of the main transcriptional targets of HIF-1 α , is the primary cytokine associated with angiogenesis. In addition to HIF-1 α and chronic hypoxia, other inducers of VEGF in SSc are interleukin-1 β , PDGF and transforming growth factor (TGF)- β (16). The VEGF family includes VEGF-A, -B, -C and -D and placenta growth factor. VEGF-A (usually referred to as VEGF) is released by fibroblasts, macrophages, endothelial cells and T cells and is involved in angiogenesis at many levels (17). The effects of VEGF are regulated by its three tyrosine kinase receptors (VEGFR-1/Flt-1, VEGFR-2/Flk-1 and VEGFR-3). VEGFR-2/Flk-1 is required for the mitotic response of endothelial cells to VEGF (18). Endostatin is the most potent inhibitor of VEGF-induced angiogenesis (17).

AO in SSc is associated with increased osteoclastogenesis and higher VEGF levels (19,20). In patients with SSc the proangiogenic VEGF-A and its receptors are paradoxically overexpressed despite insufficient angiogenesis, correlating with disease progression and fingertip ulcer development (19,20). An explanation could be the overexpression of VEGF 165b, an inhibitory splice variant of VEGF leading to insufficient angiogenesis; in patients with SSc, VEGF165b correlates with the extent of videocapillaroscopic damage and loss (21). The typical capillaroscopic changes in

SSc have been interpreted as failed angiogenic attempts following VEGF stimulation, resulting in a disturbed capillary network (19).

Ang-Tie pathway. The Ang-Tie ligand-receptor system is important in the regulation of vascular integrity and quiescence. The constitutive Ang-1/Tie2 interaction is the default vascular homeostasis control pathway, while Ang-2 acts as a dynamically regulated vessel-destabilizing cytokine (22).

The Ang-Tie ligand-receptor comprises two receptor kinases, Tie1 and Tie2, and four corresponding ligands, Ang-1, -2, -3 and -4 (23). Ang-1 inhibits VEGF-induced formation of blood vessels, the expression of adhesion molecules and TNF- α -stimulated leukocyte transmigration (22). Ang-2, a Tie2 antagonist, which is almost undetectable in endothelium at rest, is rapidly induced during activation, resulting in endothelial destabilization required for angiogenesis and/or inflammation in the presence of VEGF, while in the absence of VEGF Ang-2 causes vascular regression (24). Ang-1 is significantly decreased while serum Ang-2 is substantially elevated in SSc, particularly in patients with a 'late' nailfold videocapillaroscopy pattern, correlating with erythrocyte sedimentation rate and C-reactive protein levels, and lung involvement (25). The Ang-1/Ang-2 imbalance in patients with SSc suggests a shift toward vascular regression and angiostasis (26).

Bone perspective. Bone remodeling is a dynamic process that requires functional coordination between osteoclasts, osteoblasts and osteocytes. In SSc the generalized prevalence of osteoporosis appears to be increased, involving several humoral and cellular players (4,9).

Angiopoietin-like proteins. Angiopoietin-like proteins (ANGPTLs) are a family of proteins with structural similarities to the angiopoietins, which do not bind to Tie receptors, and have roles in lipid and glucose metabolism, inflammation, hematopoiesis and cancer (27). Of these, ANGPTL4, regulated by HIF-1 α , is able to stimulate osteoclasts even when HIF-1 α is deficient (28). Its N-terminal fragment inhibits angiogenesis, while the C-terminal fragment modulates cell adhesion. ANGPTL4 is overexpressed in the osteoclasts, synovial cells, synovial fluid and serum of patients with rheumatoid arthritis (RA), suggesting its involvement in RA erosions (29). Although arthritis is an independent predictive factor for disease progression in patients with early SSc, it seems unlikely that SSc-associated AO is due to synovial ANGPTL4 over-expression driving osteoclast-mediated bone resorption, as AO is associated with vascular involvement rather than synovitis (2,29). To date, there are no studies in which the ANGPTL4 level in SSc has been assessed. However, ANGPTL4 and ANGPTL3 share numerous features, and the level of ANGPTL3 has been found to be increased in patients with SSc, and associated with the prevalence of digital ulcers, suggesting the involvement of ANGPTL3 in the pathogenesis of SSc-associated microangiopathy (30). In this regard, clarifying the role of ANGPTLs in SSc may lead to further understanding of the complex SSc pathogenesis.

Osteoclasts. Patients with SSc and pAO exhibit increased osteoclastogenesis, associated with elevated VEGF plasma levels (20). Osteoclasts are terminally differentiated cells derived from cells with a monocyte/macrophage lineage.

Table I. HIF-regulated factors involved in angiogenesis and vascular damage in SSc.

Mediator	Vascular effects	Bone effects	Involvement in SSc	Refs.
VEGF	Increased angiogenesis	Increased osteoclastogenesis, similar to M-CSF	Increased VEGF leads to defective angiogenesis. VEGF165b inhibits angiogenesis	(10,17,21,31)
Ang-Tie	Ang-1/Tie2 regulates vascular quiescence. Ang-2 is pro-angiogenic	Bone marrow quiescence	Ang-1 is decreased and Ang-2 elevated (in late NVC patterns), with vascular regression and angiostasis	(22,25,26)
Ang-like system	Vasculoprotective, counteracts VEGF vascular signaling	ANGTPL4 is osteoclastogenic even in HIF-1 α absence	ANGPTL3 is increased and associated with digital ulcerations	(29)
Endostatin	Inhibits VEGF-induced angiogenesis	Inhibits osteoclastic bone resorption	Marker of organ damage	(32,33)
GDF15	Regulates proliferation and apoptosis of endothelial cells	Promotes osteoclastogenesis	Elevated in pulmonary hypertension	(35)
Wnt	Regulates endothelial to mesenchymal cell transition	Promotes bone formation. Inhibited by HIF-1 α and osteoblast-specific transcription factor Osterix	Wnt dysregulation is involved in fibrosis. Overexpressed and increased by hypoxia in osteoblasts	(36,39)
Notch	Angiogenesis and endothelial function regulator	Inhibits Wnt-induced osteogenesis	Overexpressed. Important in fibrosis	(36,39)
Hedgehog	Angiogenesis regulator	Osteogenesis (upstream of Wnt)	Overexpressed	(36,39)
TGF- β /BMP signaling	Regulator of angiogenesis and endothelial cell-vascular smooth muscle cell interactions	Critical regulatory functions in osteoblast differentiation and bone formation	Master regulator of fibrosis	(37,41)

Ang, angiopoietin; ANGTPL, angiopoietin-like protein; BMP, bone morphogenetic protein; CTGF, connective tissue growth factor; GDF, growth differentiation factor; HIF, hypoxia-inducible factor; M-CSF, macrophage colony-stimulating factor; NVC, nailfold videocapillaroscopy; PDGF, platelet-derived growth factor; SSc, systemic sclerosis; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

Hypoxia acts as a major stimulator of osteoclast formation and bone resorption. The secretion of VEGF-A by hypoxic osteoclasts, regulated by RANKL-mediated activation of HIF-1 α , is dependent on osteoclast size (31). However, osteoclasts express VEGFR1 (Flt-1) and, to some extent, VEGFR2 (Flk-1). Thus, local hypoxia could indirectly influence osteoclastogenesis via autocrine and paracrine secretion of VEGF under the control of HIF-1 α (31). The direct role of the VEGF-VEGFR system in osteoclastogenesis and activity was demonstrated in a study of osteopetrotic mice, characterized by the absence of functional macrophage colony-stimulating factor (M-CSF) resulting in severe osteoclast deficiency (32). The results revealed that M-CSF and VEGF play almost overlapping roles in osteoclastic bone resorption. Moreover, endostatin, a potent angiogenic inhibitor, also inhibits VEGF-stimulated osteoclastic bone resorption (33). In a study of patients with SSc, endostatin was found to be increased at all stages, while angiostatin, a platelet-derived angiogenesis inhibitor, was increased only later in the disease, and was associated with osteoarticular and lung involvement (34).

Osteocytes. Osteocytes, embedded in bone matrix and isolated from vessels, act as mechanical sensors, medi-

ated by hypoxia. Mechanical unloading, which may occur during advanced SSc, results in increased numbers of HIF-1 α -expressing osteocytes (35). Hypoxic osteocytes positively regulate osteoclastic differentiation through the secretion of growth differentiation factor 15, regulated by HIF-1 α (36). Hypoxia also decreases sclerostin secretion by osteocytes during bone remodeling (22). It may be hypothesized that osteocytic hypoxia and mechanical unloading may both be present in patients with SSc and severe hand involvement, contributing to AO.

Osteoblasts. The osteoblast has a central role in the control of VEGF-mediated angiogenesis in bone. Osteoblasts express VEGF and both VEGF-A receptors, which are upregulated by hypoxia. The overexpression of HIF-1 α by osteoblasts leads to increased angiogenesis and osteogenesis, coupled by osteoblast-generated VEGF (37). Wnt signaling is osteogenic by promoting mesenchymal stem cell differentiation, and its inactivation leads to osteoporosis (37). Also, the endothelial-myofibroblast transition involves the canonical Wnt and Notch signaling pathways, and dysregulated Wnt signaling is involved in the pathogenesis of SSc-associated fibrosis (37). The Wnt canonical pathway is activated by

TGF- β (38). The involvement of Wnt in SSc-related AO is currently unclear, but HIF-1 α and the osteoblast-specific transcription factor Osterix in osteoblasts synergistically inhibit the Wnt pathway (39). Bone morphogenic proteins (BMPs) are modulators of the Wnt pathway, while sclerostin and dickkopf-1 are endogenous Wnt pathway antagonists (37,40). Crosstalk exists between the Wnt pathway and other signaling pathways, including the Notch signaling pathway. Wnt and Notch are overexpressed in SSc (37,38). Notch pathway activation inhibits Wnt-induced osteogenesis (37). Notch signaling is activated in SSc, playing an important role in fibrosis (40), but its contribution to AO is not known. However, in Hajdu-Cheney syndrome, a rare disease evolving with AO due to Notch2 gain-of-function mutation, osteoclast hyperactivation along with endothelial impairment are involved (41).

TGF- β /BMP signaling has critical regulatory functions in osteoblast differentiation and bone formation, in addition to angiogenesis and endothelial cell-vascular smooth muscle cell interactions, and TGF- β /BMP signaling is the master regulator of fibrosis in SSc (42). Connective tissue growth factor (CTGF) negatively regulates BMP-2-induced signaling and osteoblast differentiation, and in SSc CTGF is profibrotic, along with TGF- β (43).

The main hypoxia-activated participants that are possibly involved in SSc-related AO and their effects on the endothelial and bone cells are summarized in Table I.

3. Conclusions

In this review the critical role of the HIF-1 α /VEGF signaling pathway in regulating osteoclastic bone-resorption and angiogenesis is highlighted, providing evidence that increased osteoclastogenesis and higher VEGF levels may contribute to AO in patients with SSc. Cells of the osteoblast lineage also have important roles in angiogenic-osteogenic coupling. Although in the complex pathogenesis of AO osteoclast resorption appears to be the main mechanism, the impairment of osteoblastic bone formation cannot be ruled out. There are several aspects of the pathogenesis that remain unclear and require clarification, in addition to the association between calcinosis and AO. However, the research in this field might help to limit the acral changes, which contribute to the disability associated with SSc.

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