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Review

The emerging role of endothelin-1 in the pathogenesis of subchondral bone disturbance and osteoarthritis

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SUMMARY

Mounting evidence suggests reconceptualizing osteoarthritis (OA) as an inflammatory disorder. Trauma and obesity, the common risk factors of OA, could trigger the local or systemic inflammatory cytokines cascade. Inflammatory bone loss has been well documented; yet it remains largely unknown about the link between the inflammation and hypertrophic changes of subchondral bone seen in OA, such as osteophytosis and sclerosis. Amid a cohort of inflammatory cytokines, endothelin-1 (ET-1) could stimulate the osteoblast-mediated bone formation in both physiological (postnatal growth of trabecular bone) and pathological conditions (bone metastasis of prostate or breast cancer). Also, ET-1 is known as a mitogen and contributes to fibrosis in various organs, e.g., skin, liver, lung, kidney heart and *etc.*, as a result of inflammatory or metabolic disorders. Subchondral bone sclerosis shared the similarity with fibrosis in terms of the overproduction of collagen type I. We postulated that ET-1 might have a hand in the subchondral bone sclerosis of OA. Meanwhile, ET-1 was also able to stimulate the production of matrix metalloproteinase (MMP)-1 and 13 by articular chondrocytes and synoviocytes, by which it might trigger the enzymatic degradation of articular cartilage. Taken together, ET-1 signaling may play a role in destruction of bone-cartilage unit in the pathogenesis of OA; it warrants further investigations to potentiate ET-1 as a novel diagnostic biomarker and therapeutic target for rescue of OA.

Introduction

Osteoarthritis (OA) is the most common of age-related degenerative joint disorders. It is a leading cause of pain and disability among older adults. OA is a whole joint disorder with low-grade inflammation, afflicting articular cartilage, subchondral bone, synovium, ligaments and joint capsule. The hallmark of OA is the degradation of articular cartilage. The integrity of articular cartilage relies on the interplay with the other joint tissues, in particular subchondral bone[1]. On the basis of hypertrophic changes of subchondral bone such as osteophytosis and sclerosis, OA is differentiated from the other types of arthritis, e.g., rheumatoid arthritis (RA).

Recently, mounting evidence suggests that OA should be conceived as an inflammatory disease rather a simple wear-and-tear problem[2]. The complements system was found to play a central role in the initiation and deterioration of cartilage damages[2]. Joint trauma and incurred joint instability could activate local inflammatory response. Transforming growth factor-β1 (TGF-β1), a key inflammatory mediator, was activated at subchondral bone in response to anterior cruciate ligament transection (ACLT) to initiate the onset of OA in a mice model[3]. Obesity is a recognized risk factor of OA[4]. As part of metabolic syndrome (MetS), diabetes was also proposed as an emerging independent risk factor of OA[5]. We once observed a significant bone loss at subchondral plate in OA patients with the comorbidities, i.e., hypertension and diabetes[6]. Systemic inflammation is a hallmark of MetS. Inflammatory bone loss has been documented in RA[7], yet the biological link between inflammation and the disturbances at OA subchondral bone, in particular its hypertrophic or sclerotic changes, remains largely unknown.

Actually, osteoblasts derived from sclerotic bone of OA patients exhibited high profile of inflammatory cytokines including TGF-β1,

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prostaglandin E2 (PGE_2), interleukin-1β (IL-1β), interleukin-6 (IL-6) and *etc.*[8]. Amid a cohort of inflammatory cytokine mediators, endothelin-1 (ET-1) is known to mediate physiological and pathologic bone formation[9]. In addition, ET-1 has been implicated in the degradation of articular cartilage in either inflammatory or degenerative arthritis[10–15]. Meanwhile, high level of plasma ET-1 was also identified in the obese individuals and patients with hypertension and diabetes[16]. All data pointed in the direction that ET-1 could be one of candidate inflammatory factors to govern the hypertrophic changes of subchondral bone, as a sequel of local and systemic inflammation, in the pathogenesis of OA. We therefore aimed to identify the information gap regarding the role of ET-1 in OA subchondral bone disturbance by the integration and interpretation of the existing data about ET-1 signaling in inflammation, arthritis, the physiological and pathologic bone formation and *etc.* It would provide a new insight into molecular mechanism underlying the destruction of bone–cartilage unit and shed light on the discovery of therapeutic targets for OA.

ET-1, inflammation and fibrosis

ET-1, a 21-amino acid peptide, was first discovered as a potent vasoconstrictor in 1988[17]. It has two structurally similar G protein-coupled receptors, endothelin type A receptor (ETAR) and type B receptor (ETBR). It is received that ETAR is responsible for transducing the most of biological effects of ET-1 while ETBR serves primarily as a clearance receptor[18]. ET-1 is synthesized as preproET-1 that is cleaved to form the precursor big ET-1 or proET-1. Endothelin converting enzyme-1 converts big ET-1 to form the active peptide ET-1[19]. Big ET-1 only has 1/100 of the potency of mature ET-1. Extracellular conversion to ET-1 could elicit sufficient stimulus for biological processes subsequently[18].

ET-1 and nitric oxide (NO) act against each other to maintain vascular homeostasis and the balance of vasoconstriction and vasodilation[20]. Hypertension could be a result of the disturbed balance between ET-1 and NO[21]. The overexpression of ET-1 specifically in endothelial cells using *tie-1* promoter could lead to systemic hypertension with altered vascular reactivity in a mouse model[22]. A reduction of systemic blood pressure in patients with essential hypertension by ET-A receptor antagonists further demonstrated the link between ET-1 signaling and hypertension[23]. Moreover, insulin has been shown to induce ET-1 production at the transcriptional level[24], and ET-1 may involve in the vicious cycle of insulin resistance in the pathogenesis of diabetes[25].

Also, ET-1 may contribute to the complications of hypertension or diabetes such as the fibrosis of heart, lung, kidney and skin by activating the inflammatory and fibrotic signaling pathways[26,27]. The severity of fibrosis appeared in an association with the level of local ET-1[28]. Growing bodies of evidence suggested that ET-1 cooperated with TGF-β1, a known pro-fibrotic factor, in the pathogenesis of fibrosis in various tissues[29–31]. Blockade of endothelin signaling either pharmaceutically (ETAR or ETBR antagonists or neutralizing antibodies) or genetically (ETAR or ETBR siRNA) could decrease the secretion of TGF-β by fibroblasts and attenuate the fibrosis phenotype[26]. TGF-β1 also promoted ET-1 production in a Smad2/3-dependent fashion in skin fibroblasts[29]. In the fibrotic lung fibroblasts, TGF-β1 activates ET signaling through type I receptor and then downstream ALK5/c-Jun N-terminal kinase (JNK)/Ap-1 signaling that is independent of Smad proteins[30]. The dual ETA/ETB receptor antagonist, e.g., Bosentan, could interrupt the constitutive JNK activation in fibrotic fibrolasts, providing the evidence of an autocrine endothelin loop of ET-1 in its fibrotic effects. In addition, ET-1 may exert its fibrotic effects by activating a tissue factor/thrombin amplification loop, which is responsible for the production of connective tissue growth factor (CTGF)[31]. Last but not least, the endothelial mesenchymal transition (EndMT) process was also implicated in the ET-1-mediated fibrosis[32]. Taken together, targeting the axis of TGF-β1/ET-1 signaling is likely to be of benefit in battling with fibrotic disorders.

ET-1 and arthritis

ET-1 has been implicated as a major informatory mediator in various autoimmune diseases such as RA[33] and scleroderma[34] (Table I). It was reported that both local (synovial fluid) and systemic (serum) levels of ET-1 were significantly higher in RA patients than in healthy subjects[10]. Synovial macrophage-like cells could produce ET-1[35] that interacted with the other inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-1β to regulate the adhesion proteins production, e.g., intercellular adhesion molecule-1, vascular cell adhesion molecule-1, CD106 and CD44 in cultured synovial fibroblasts[36,37]. It supports a notion that ET-1 plays a role in the inflammatory responses of synovitis by recruiting neutrophil and endothelial cells to infiltrate the inflamed tissue in arthritis[38]. Moreover, the elevated level of local ET-1 was also linked up to the extra-articular manifestations of RA, e.g., hypertension[39]. IL-6, the most abundant pro-inflammatory cytokines in the serum and synovial joints of RA, could directly induce the preproET-1 mRNA expression in the fibrotic kidney with angiotensin II-induced hypertension[27].

ET-1 could exert its direct effects on articular chondrocytes as well as the synovial inflammation and fibrosis. Articular chondrocytes themselves rarely express ET-1 unless the natural aging process[13] or diseased conditions[14,15]. In addition, the expression level of ET receptors on articular chondrocytes was also affected by the age as well as various growth factors and cytokines including platelet derived growth factor-BB (PDGF-BB), TGF-α, TNF-α, IL-1β and *etc.*[40,41]. The ET-1- binding sites on articular chondrocytes were predominantly of ETAR[41]. The number of ET-1 binding sites on the aged chondrocytes was approximately twice as many as the

Table I
Endothelin signaling in various types of arthritis and other musculoskeletal disorders

Authors	Type of arthritis	Specimen	Findings
Nahir AM *et al.*[12]	RA, OA	Synovial fluid	Endothelin level in synovial fluid of RA was similar to in OA.
Haq A *et al.*[10]	RA	Serum and synovial fluid	ET-1 level in serum and synovial fluid of RA patients was higher than normal.
Yoshida H *et al.*[11]	RA	Synovial cells	Synovial macrophage-like type A cells could produce ET-1.
Iwabuchi H *et al.*[36]	RA, OA	Synovial fibroblasts	TNF-α up-regulated ICAM-1 in RA and OA fibroblasts while ET-1 inhibited this process.
Miyasaka N *et al.*[87]	Inflammatory arthritis	Synovial fluid	ET-1 in synovial fluid was higher than serum.
Roy-Beaudry M *et al.*[15]	OA	Articular chondrocyte	ET-1 induced MMP-1 and 13.
Manacu CA *et al.*[14]	OA	Articular chondrocyte	ET-1 caused NO, MMP1 and MMP13 overexpression.
Wei Yuan *et al.*[45]	Lumbar disc degeneration	Cells from endplate	ET-1 increased MMP-1 and MMP-13, decreased TIMP-1, and induced NO.

Abbreviation: ET-1, endothelin 1; RA, rheumatoid arthritis; OA, osteoarthritis; TNF-α, tumor necrosis factor-alpha; ICAM-1, Intercellular Adhesion Molecule 1; MMP-1/13, metalloproteinase 1/13; TIMP-1, tissue inhibitor of metalloproteinase-1.

younger ones[41]. It was shown that the responsiveness of articular chondrocytes to ET-1 stimuli *in vitro* could be enhanced in the presence of PDGF-BB, TNF-α, and IL-1β, but diminished with TNF-α[40,41]. It strongly suggested that ET-1 was involved in regulating the cellular metabolism in health, and presumably in the aged and disease chondrocytes. Local level of ET-1 in OA joint was comparable to RA[12]. The elevated ET-1 could provoke the expression of matrix metalloproteinase (MMP)-1 and MMP-13 by osteoarthritic chondrocytes *in vitro*[15,16], the major enzyme to decay the cartilaginous matrix in the process of OA[14,15]. We further observed that overexpressing endothelial ET-1 exaggerated hypertrophic differentiation of articular chondrocytes with thickening calcified cartilage that caused the structural damage to hyaline cartilage in a transgenic mouse model[42]. On the other side, ET-1 could stimulate the proteoglycan and collagen production by articular chondrocyte in a dose- and time-dependent manner[43,44]. The stimulatory effect of ET-1 in the first 24-h were gradually decreased and finally inhibited with prolonged incubation. NO mediated such inhibition of ET-1 effects. Very recently, local level of ET-1 was also linked up to the dysfunctional disc cells in the degeneration of lumbar intervertebral disc[45].

ET-1 may have a hand in mediating the inflammation-induced pain, a major complaint from arthritis patients. It was found that the subcutaneous injection of ET-1 could produce pain behavior and activate the nociceptive C-fibers in a dose–dependent manner[46]. Use of ETAR antagonist or knockout of ETBR significantly reduced both the subcutaneous and joint pain in rats[46,47]. It has been documented that ETAR antagonists, in conjunction with bradykinin receptor antagonists, were able to lessen the pain and protect the morphology of OA knee joint in an ACLT mouse model[48]. However, not all of patients with the radiologic OA are symptomatic, in the other words, present the painful joints. The poor correlations of the common findings from x-radiograph with pain pose a big challenge in the management of OA[49]. The mechanical or inflammatory stimuli activated the expression of nerve growth factor (NGF) by articular chondrocytes and stimulated nerve in-growth at the osteochondral junction, which may involve in the arthritic joint pain[50,51]. It has been known that ET-1 could control the NGF production in cardiac innervation[52]. Thus, we postulated that ET-1 might play a role in mediating pain and nerve in-growth at the osteochondral junction in OA. If so, ET-1 would be a promising biomarker for OA pain.

By contrast, the exact role of ET-1 in the disturbance of subchondral bone in arthritic joint remains largely unknown. Unlike the common x-radiographic findings, the edema-like changes under magnetic resonance imaging, i.e., so-called bone marrow lesions (BMLs), were found in an association with the severity of pain in OA[49]. BMLs were characterized histologically by the sclerotic bone but less mineralized[53]. Osteoblasts derived from the sclerotic bone of OA overproduced collagen type I with the aberrant composition of alpha1 and alpha2 chains, and subsequently led to poor mineralization[54]. High level of inflammatory cytokines or mediators including TGF-β1, IL-1β, IL-6 and PGE_2 may constitute the defective osteoblasts[8]. The defective osteoblasts could alter the phenotype of normal articular chondrocytes towards hypertrophic differentiation by suppressing the expression of parathyroid hormone related protein (PTHrP) and increasing the level of MMP-13[55]. Neutralizing the activities of such inflammatory cytokines or mediators such as TGF-β1 could restore aberrant overproduction of collagen type I by defective osteoblasts *in vitro*[54], and attenuate the severity of OA *in vivo*[3]. Given the similarity between the fibrotic and sclerotic processes in terms of type I collagen overproduction, we postulated that the axis of TGF-β1/ET-1 signaling, the known key regulators in fibrosis, may play a role in the sclerotic changes of OA subchondral bone.

ET-1 in physiological and pathological bone formation

Role of ET-1 in the maintenance of bone homeostasis and regulation of osteoblastic function has been widely discussed (Table II). It was found that mice deficient in ET-1 had the craniofacial abnormalities of poorly developed mandibles and lower bone mass[56,57]. It suggested that ET-1 was involved in intramembranous ossification process of flat bone. When ETAR was specifically inactivated in the osteocalcin-positive osteoblasts, trabecular bone mass and volume in long bone, i.e., tibia, were significantly decreased in mice[9]. It indicated the regulatory role of ET-1 in the postnatal development of long bone, possibly involving an endochondral ossification process. Moreover, the male and female mice showed different responses to the knockout of ETAR. Such gender difference did imply the interactions of sex hormone, particularly androgen, with ET-1 signaling in mediating bone metabolism. It warrants the further investigations to look for the direct evidence from the castration and ovariectomy models.

In agreement with the *in vivo* data, the most of *in vitro* studies demonstrated that the exogenous ET-1 promoted the differentiation and proliferation of osteoblast precursors[58–60]. The effects of ET-1 stimuli transduced through an integral membrane protein, connexin-43 for osteoblast differentiation[60]. Then it could increase the mRNA synthesis of osteopontin and osteocalcin, the markers of mature osteoblasts, and stimulate the production of alkaline phosphatase (ALP) and type I collagen[61]. As a potent regulator of bone metabolism, the exogenous ET-1 might also interfere with the mineralization process of osteoblasts[62–64]. But the results generated from the previous studies remain questionable as the MC3T3-E1 cell lines and the transformed osteoblasts in osteosarcoma being tested tended to be over-mineralized and they might not be a good model to investigate the physiologic bone mineralization process.

Compared to osteoblasts, the information about ET-1 function in osteoclasts is limited and ambiguous. ET-1 was detected in the membrane and cytoplasm of osteoclasts by immunostaining[65]. It implies that osteoclasts might be one of the target cells for ET-1 stimuli. By adding ET-1 (2–8 nM) to osteoclasts culture, the osteoclasts-mediated bone resorption was decreased and the cell motility was also inhibited[66]. The opposite results were reported in another study on orthodontic tooth movement. It was found that ET-1 could increase osteoclastic bone resorption *via* ETAR[67,68]. ET-1 was the predominant form of endothelins isoforms in the late stage of orthodontic tooth movement[69]. The nanomolar level of ET-1 was able to stimulate osteoclastic activity. Endothelial cells and their producing ET-1 in bone may regulate bone remodeling by controlling osteoclastic activities and bone resorption.

ET-1 signaling also contributed to the pathological bone formation, e.g., osteoblastic metastasis in prostate cancer[70]. High level of ET-1 produced by the prostate cancer cell lines (DU-145 and LNCaP) could stimulate BMP-induced bone growth *in vitro*[70], and bone metastasis *via* interacting with ETAR[58,71]. The ET-1 signaling antagonists have been proposed to control the progression of bone metastasis in prostate cancer[72].

The extracellular molecular mechanism underlying ET-1-mediated bone formation was possibly involving the autocrine or paracrine activation of Wnt signaling that is essential for osteoblast proliferation, differentiation and bone development (Fig. 1). Dickkopf homologue 1 (DDK1) is a selective inhibitor of the Wnt signaling pathway and its transcription rate could be suppressed by ET-1 in calvarial organ culture[73]. On the other hand, recombinant DDK1 could also inhibit the ET-1-mediated osteoblast proliferation and new bone formation[73]. ET-1 interacted with DKK1 in activation of Wnt signaling and mediating new bone formation. Microarray analysis has uncovered several prominent expression motifs activated by ET-1 in osteoblasts[71]. For example, OPG (osteoprotegerin)/

Table II
Endothelin signaling in regulation of osteoblastic function

Authors	Source of osteoblasts	ET-1 stimulation	ET receptor blockade			Findings
			Type A	Type B	Both	
C. H. Kasperk *et al.*[61]	Human trabecular bone	10^{-1}–10^{-5} pg/ml	1–100 pg/ml	/		Increase ALP, collagen type I production, increase bone cells proliferation
Akiyoshi Someya *et al.*[59]	MC3T3-E1	10^{-6}–10^{-13} M		/		ET-1 increase the intracellular Ca^{2+} concentration, DNA synthesis and cell number, as well as the decrease in ALP activity
H.P. von Schroeder *et al.*[58]	Calvarial cell from fetal litters of 21-day pregnant Wistar rats	10^{-6}–10^{-10} M		/		ET-1 stimulates osteoblastic proliferation and differentiation.
Corinne Niger *et al.*[60]	hFOB 1.19	10^{-7}–10^{-8} M		/		Cx43 expression level could influence the action of ET-1 on differentiation of human Ob.
Yoshiharu Hiruma *et al.*[62]	MC3T3-E1	10^{-7}–10^{-12} M	BQ123 10^{-6} μM	/		ET-1 decreases the expression of mRNA of osteocalcin, decrease deposition of Ca^{2+}, increased the production rate of IP_3
Hing-Chung Lam *et al.*[64]	HTb-96 (human osteosarcoma)	/		/		Human transformed osteoblast (HTb-96) can produce ET-1.
Mitsuhiro Shioide *et al.*[63]	ROS17/2.8 (Rat Osteosarcoma)	10^{-7}–10^{-9} M		/		Enhancement of the abundance of osteopontin and osteocalcin mRNAs in a time-dependent manner.
Agui T. *et al.*[76]	Bone marrow cells from femoral bone of male F344/Tj	10^{-7}–10^{-11} M		/		ET regulates IL-6 production by bone marrow derived stromal cells.
Sherrie L. Perkins *et al.*[77]	MC3T3-E1, primary murine osteoblast	0.01–100 nM	BQ123 1 μM	/		ET-1 enhanced IL-6 production.
Matsuno M *et al.*[80]	MC3T3-E1	10^{-6}–10^{-9} M	BQ123 22 nM	BQ788 1.2 nM	/	ET-1 induces IL-6 production *via* PKC
H. Kawamura *et al.*[78]	MC3T3-E1	10^{-7}–10^{-10} M		/		PKC-dependent p42/p44 MAP kinase activation is involved in ET-1-induced IL-6 synthesis in osteoblast-like cells.
Haruhiko Tokuda *et al.*[82]	MC3T3-E1	3 nM, 0.1 μM		/		EGCG reduces ET-1 induced IL-6 synthesis in MC3T3-E1 cells and primary cultured mouse osteoblasts
Haruhiko Tokuda *et al.*[81]	MC3T3-E1	0.1 μM		/		Rho-kinase is involved in regulation of ET-1-stimulated IL-6 synthesis through p38 MAP kinase activation
Allison M. Kitten *et al.*[88]	Fetal rat calvarial	/	BQ123 1 μM	BQ788 1 μM	TAK-044 100 nM	BMP-7 enhances ET-1 production but not TGF-bata 1
Hiroko Masukawa *et al.*[89]	MC3T3-E1	0.01–100 nM		/		ET-1 causes activation of PKC through both phosphoinositide and phosphatidylcholine hydrolyzes and lead to Pi transport
W. Windischhofer *et al.*[90]	MC3T3-E1	0.1–100 nM		/		ET-1–induced expression of PGHS-2 mRNA requires activation of the Rho family of G proteins and p38 MAPK
David E. Semler *et al.*[91]	MMR-106 (Rat Osteosarcoma)	10^{-7}–10^{-11} M	BQ123 10^{-5}–10^{-9} M	/	/	ET-1 evokes Ca^{2+} transient
Yoh Takuwa *et al.*[85]	MC3T3-E1	10^{-7}–10^{-11} M		/		ET-1 cause Ca^{2+} mobilization and PK C activation
Iris Schvartz *et al.*[92]	MC3T3-E1	10^{-7} M		/		Activation of tyrosine kinase phosphorylation
A. Suzuki *et al.*[93]	MC3T3-E1	0.1 μM	BQ123 22 nM–2.2 μM	BQ788 1.2 nM–120 nM		Three intracellular pathways: (1) phosphoinositide hydrolysis; (2) phosphatidylcholine hydrolysis; and (3) arachidonic acid release are mediated by ETAR

RANKL (receptor activator of nuclear factor kappa-B ligand)/RANK axis tightly controls osteoclastogenesis and maintains bone homeostasis[74]. ET-1 stimulated the production of OPG not only in atherosclerosis but also osteoblastic metastasis of tumor[71,75]. The interplays between ET-1 and the other inflammatory/pro-fibrotic factors such as uroplasminogen activator, uroplasminogen activator receptor, plasminogen activator inhibitor 1, TGFβ1, IL-6, vascular endothelial growth factor and CTGF have also been reported in bone marrow stromal cells and osteoblasts[58,71,76–82].

The intracellular molecular mechanism underling ET-1 mediated bone formation may include the calcineurin/Nuclear factor of activated T-cells (NFAT) pathway[71]. It was found that NFATc1 underwent nuclear translocation by the stimuli of ET-1 in either the committed and differentiated MSCs towards osteoblasts[71]. ET-1 could increase the intracellular calcium levels through ETAR. The activated calcineurin by ET-1 and the calcium influx induced the dephosphorylation and nuclear translocation of NFAT family members, which led to the formation of a transcriptional complex

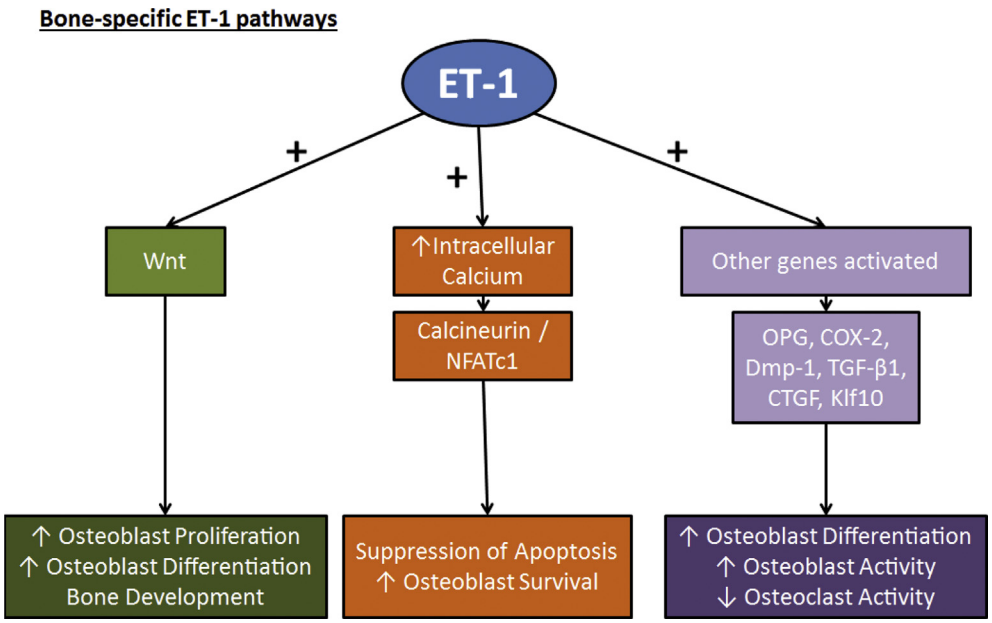

Fig. 1. ET-1 signaling pathways specific to osteoblasts. ET-1 activates the Wnt pathway leading to osteoblast proliferation and differentiation; the calcineurin/NFATc1 pathway leading to suppression of apoptosis, which encourages cell survival; as well as other genes, that together increases osteoblast proliferation and differentiation while inhibits osteoclast activity.

and transcriptional activation of NFAT-controlled genes for the suppression of apoptosis[71]. ET-1 might support the survival of osteoblasts by activation of the calcineurin/NFAT signaling pathway. The Rho family of G proteins, protein kinase C (PKC)[80], inositol 1,4,5-trisphosphate (IP_3)[76] and p38 mitogen-Activated PK (MAPK) *etc.*[78,79], also constituted the intra-cellular ET signaling proteins. First, ET-1/ETAR signaling is activated *via* G protein coupled receptor to phospholipase C (PLC) and phospholipase D (PLD)[83,84]. PLC or PLD hydrolyzes phosphatidylinositol 4,5-bisphosphate to form water-soluble IP_3 and diacylglycerol (DAG)[19], which in turn increases intracellular calcium concentration[85]. DAG and elevated intracellular calcium concentration then activates PKC[19]. Second,

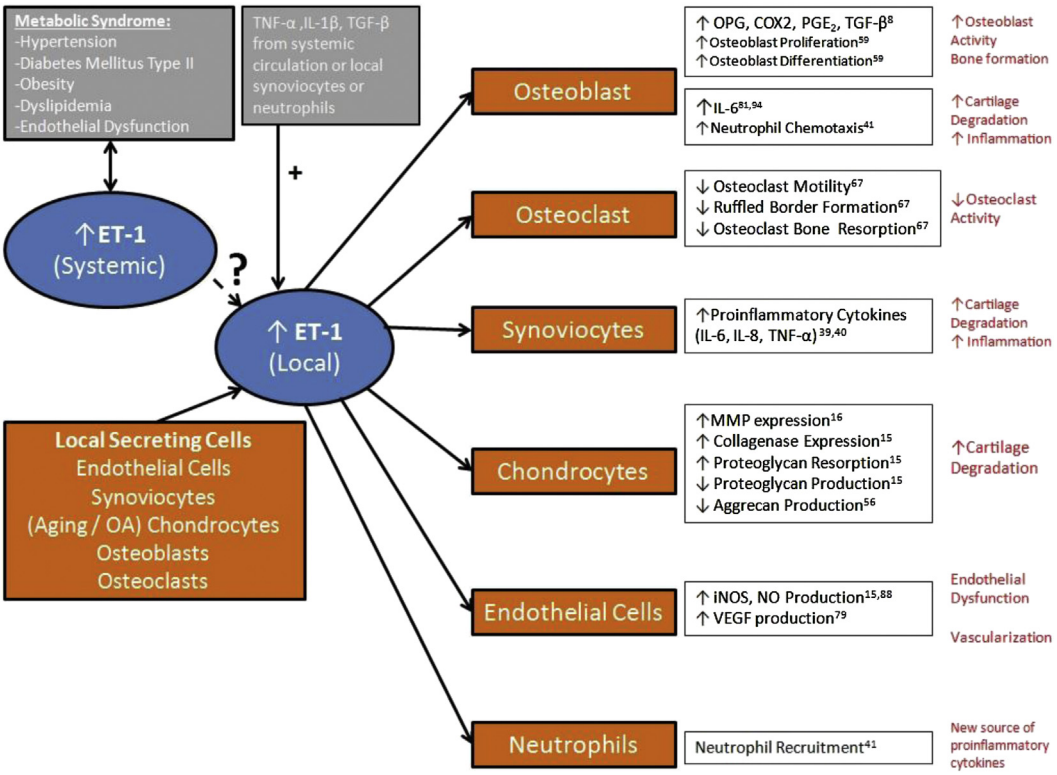

Fig. 2. The emerging role of ET-1 on the osteoarthritic joint. ET-1 induces osteoblastic proliferation and differentiation while inhibiting osteoclastic activity. ET-1 also increases secretion of proinflammatory cytokines and neutrophil recruitment. ET-1 acts on chondrocytes to increase MMPs production and alterations in proteoglycan production and resorption balance, leading to cartilage degradation and destruction. ET-1 also perturbs NO production in endothelial cells and VEGF secretion, which can result in endothelial dysfunction and angiogenesis.

MAPK is another downstream intracellular signaling protein of ET-1[86]. ET-1 was shown to activate MAPKs such as extracellular signal regulated kinases 1 and 2 (ERK1/2), p38 MAPK and c-JNKs in cardiomyocytes, fibroblasts, glomerular mesangial cells and vascular smooth muscle cells. ET-1 might induce VGEF expression through activation of p38 MAPK in osteoblasts[79]. Last, PI3-K acts as a protein/lipid kinase and phosphorylates membrane-localized phosphatidylinositides to form secondary messenger phospholipids and to activate PK B (PKB, also known as Akt) by binding to ETAR. ET-1 could induce PKB activation in cardiomyocytes, myofibroblasts, vascular endothelial cells and smooth muscle cells as well as OA chondrocytes. PKB activation often leads to changes in the expression of genes involved in cell survival (BAD, caspases, GSK3) and regulation of protein synthesis (eIF4E, eIF4E-binding protein 1). In brief, ET-1 plays a role in regulation of the physiological and pathological osteoblastic function mainly through ETAR, involving the activation of the extracellular (DKK1, OPG) and intracellular signaling proteins (calcineurin/NFAT and MAPK).

Perspectives

ET-1 signaling is proposed to afflict all types of cells in the joint, e.g., articular chondrocytes, osteoblasts and synoviocytes in the pathogenesis of OA (Fig. 2). First, ET-1 may exert an anabolic effect on subchondral bone by stimulating osteoblasts and inhibiting osteoclasts. Uncoupling effects of ET-1 on bone remodeling might contribute substantially to the disturbance of subchondral bone in OA. Second, ET-1 may trigger the enzymatic degradation of articular cartilage. Third, ET-1 could recruit inflammatory cells into inflamed joint such as neutrophils and macrophages that might account for synovium thickening and synovitis in OA. The origins of the elevated ET-1 could be from either local or systemic inflammation. The abnormal mechanical loads might induce the repetitive microdamage and an inflammatory response with local production of ET-1. The co-morbidities such as obesity, hypertension and diabetes may also contribute to high level of ET-1 in the situation of systemic inflammation. TGFβ1/ET-1 signaling has been well studied in fibrotic process. It warrants further investigation whether TGFβ1/ET-1 signaling also plays a role in osteoblast dysfunction and subchondral bone sclerosis in the pathogenesis of OA.

Authors' contribution

Sin prepared the first draft of this review article and Tang supplemented the paragraphs and summarizing tables about the *in vitro* studies of ET-1 on bone cells. Wen, Chung and Chiu proposed the idea, revised the manuscript critically and approved the final version to be published.

Competing interests

The authors declare that they have no competing interests.

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