Mechanism of cancer-induced bone destruction: An association of connective tissue growth factor (CTGF/CCN2) in the bone metastasis

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KEYWORDS
Bone destruction; Bone microenvironment; Osteoclast; CTGF/CCN2

Summary  Connective tissue growth factor (CTGF/CCN2) is a member of the CCN family, a novel class of extracellular signal modulators. CCN2 is composed of four conserved modules connected in tandem, each of which is rich in cysteines and highly interactive with other molecules. CCN2 has various biological functions, being active in developmental processes including angiogenesis, chondrogenesis, and osteogenesis. Recently CCN2 has gained more clinical interest due to its role in cancer-induced bone destruction. In this article, the role of CCN2 in bone-destroying events as an organizer of the microenvironmental cell society is comprehensively described, and a brief summary of the recent findings on regulatory factors involved in tumor-induced bone disease is given.

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1. Introduction

Bone has been identified as a site of metastasis of cancer cells of several common human malignancies, including breast, prostate, multiple myeloma, and oral cancer [1–5]. The clinical consequences of cancer-induced bone destruction include a worse prognosis, physical damage by bone resection, a high morbidity rate, intractable bone pain, pathological fractures, and hypercalcemia [6]. Localization of tumor cells within the bone leads to the production of tumor-associated factors either synthesized directly by the tumor cell itself or as a result of tumor/stromal interactions. These tumor-associated factors converge on the pre-osteoblast or stromal cell to cause an increase in the level of receptor activator of nuclear factor kappa ligand (RANKL) and/or a decrease in that of osteoprotegerin (OPG), which ultimately results in activation and survival of osteoclasts, with osteolytic lesions being the result [7]. Osteolysis (process of bone resorption) then leads to the release of growth factors derived from bone, including transforming growth factor-β (TGF-β), insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), and bone morphogenetic proteins (BMPs) [7,8]. These factors increase the production of tumor-associated factors or promote tumor growth directly. Thus, tumor cell proliferation and production of tumor-associated factors through the signaling of these pathways are promoted, and the cycle continues.

Connective tissue growth factor (CTGF/CCN2) belongs to the CCN family [9], which consists of six members: CCN1 (Cyr61), CCN2 (CTGF), CCN3 (NOV), CCN4 (WISP-1), CCN5 (WISP-2), and CCN6 (WISP-3) [10–12], all of which possess an NH2-terminal signal peptide indicative of their secreted-protein nature. These proteins comprise distinct modules in their structure, i.e., the insulin-like growth factor (IGF)-binding protein-like module (IGFBP), von Willebrand factor type C repeat (VWC), thrombospondin type-1 repeat (TSP1), and C-terminal module (CT), except for CCN5, which lacks the CT module. With these modules, the CCN2 protein interacts with a number of extracellular molecules. The IGFBP motif is responsible for binding IGF [13], albeit studies with CCN2 have demonstrated that the interaction of CCN2 with IGF has a much lower affinity than that of authentic IGFBPs [14]. The VWC motif binds to integrin αvβ3 [15] and has been implicated as a binding site for BMP-4 and transforming growth factor-β (TGF-β) family members, this binding modulating their activity [16]. The TSP-1 motif is involved in binding to integrin α6β1, αvβ3 [15], LRP1 [17], and VEGF [18]. Finally, the CT motif binds integrin αvβ3 and cell-surface heparan sulfate proteoglycans (HSPGs) [19]. These different domains of CCN2 could be responsible for the differential signaling of its biological activities (Fig. 1A).

This review summarizes research on a new molecule, CCN2, and related molecules that play essential roles in the bone destruction caused by cancer.

![Figure 1](image-url)  
**Figure 1** CCN2-interacting proteins and biological functions of CCN2. (A) Interacting proteins and correspondence to modules of CCN2. Interactions with growth factors, cell-surface signal-transducing receptors, and heparan sulfate proteoglycans (HSPGs). (B) Biological functions of CCN2 in development. CCN2 is strongly expressed in the endothelial cells, mesenchymal cells, and hypertrophic chondrocytes in the growth plate of cartilage and has principal roles in development.
2. Physiological and pathological roles of CCN2

2.1. Biological function of CCN2 in skeletal development

CCN2 knockout mice die just after birth due to respiratory failure [20]. This failure is attributed to hypoplasia of the thoracic skeleton and deformity of the oral cavity (palatal cleft and shortened mandible). CCN2 knockout mice show skeletal dysmorphisms as a result of impaired chondrocyte proliferation and reduced extracellular matrix composition within the hypertrophic chondrocytic zone in the growth plate. Histologically, angiogenesis and formation of tartrate-resistant acid phosphatase (TRAP)-positive osteoclast-like cells, as well as critical protease expression in the growth plate, are impaired and accompanied by defective replacement of cartilage by bone during endochondral ossification. These results demonstrate that CCN2 is important for cell proliferation and matrix remodeling during chondrogenesis, and is a key regulator coupling extracellular matrix remodeling to angiogenesis at the growth plate. These activities are consistent with the notion that recombinant CCN2 induces chondrocyte and osteoblast differentiation and proliferation in vitro [21–23], and angiogenesis in vivo and in vitro [24,25]. The biological activities of CCN2 also include the development of Meckel’s cartilage [26] and tooth germs [27] (Fig. 1B).

2.2. Association of CCN2 in cancers

CCN2 is a secreted growth factor that can bind to integrins on the cell surface [28], and elevated CCN2 expression has been observed in breast cancers [29], pancreatic cancers [30], melanomas [31], chondrosarcomas [32] and squamous cell carcinomas [33]. Although CCN2 shows multiple roles in various cancer types, in breast tumor cells CCN2 overexpression has been linked to an increase in tumor size, lymph node metastasis [29,34], and drug resistance through up-regulation of the survival pathway [35]. CCN2 is also regarded as a central mediator of tumor angiogenic factor in certain malignancies [36–38]. It should be noted that CCN2 is one of the contributors to bone metastasis, as it converts low-metastatic breast cancer cells to high-metastatic ones in the collaboration with other factors [39,40]. Furthermore, CCN2 gene was significantly overexpressed in overt metastatic tumor cells as compared to disseminated tumor cells in the bone marrow of breast cancer patients by CT-guided bone metastasis and bone marrow biopsy [41]. Fig. 2A and B shows representative radiographs and immunohistochemical analysis of hind limbs from mice 25 days after MDA-MB-231 cells intracardiac inoculation. Obvious osteolytic lesions were present in mice that had received control IgG, whereas very few metastatic lesions were present in the mice treated with the anti-CCN2 Ab at a dose of 100 μg/mouse twice per week throughout the experiment (Fig. 2A). CCN2 and PTHrP were strongly expressed in cancer cells that had invaded the bone matrix, and these CCN2-expressing cells also expressed PTH1R (Fig. 2B). Fig. 2C illustrates a representative radiographic pattern of invasive bone destruction observed in a patient with oral squamous cell carcinoma in the mandibular region. CCN2 was abundantly produced by the tumor cells that had invaded the bone matrix (Fig. 2D) of note, up-regulation of CCN2 in mandible oral squamous cell carcinoma was associated with increased bone destruction [33]. These data suggest that CCN2 can be considered a diagnostic marker and target for treatment in oral osteolytic mandibular squamous cell carcinoma.

3. The role of CCN2-regulating factors in bone resorption

3.1. Parathyroid hormone-related protein (PTHrP) induce CCN2 expression in bone metastatic cancer cells

Parathyroid hormone-related protein (PTHrP) plays a vital role in the development of the embryonic skeleton and other tissues. When it is produced in excess by cancers, it can cause hypercalcemia; and its local production by breast cancer cells has been implicated in the pathogenesis of bone metastasis in that disease. Localized production of PTHrP by cancer cells in such lesions was shown to promote the survival and proliferation of cancer cells and osteolysis in a mouse model [42]. PTHrP induces both the production of RANKL and down-regulation of OPG production by osteoblasts, thereby stimulating osteoclastogenesis [43,44]. Type I PTH/PTHrP receptor (PTH1R) expression was specifically observed in cancer cells producing PTHrP and CCN2 invaded the bone marrow (Fig. 2B) and PTHrP strongly upregulated CCN2 in MDA-MB-231 cells in vitro [45]. CCN2 was critically involved in osteolytic metastasis and was induced by PKA- and PKC-dependent activation of ERK 1/2 signaling by PTHrP [45].

3.2. Transforming growth factor-β (TGF-β) is one of the most potent inducer of CCN2

Transforming growth factor-β (TGF-β) is by far the most abundant cytokine in bone, 200 μg/kg tissue, and must be considered as a central player in bone turnover [46] and potentially able to couple bone resorption with bone formation [47,48]. Restricted to the bone environment, target cells include cancer cells as well as osteoblasts, osteoclasts, their precursors bone marrow and stromal cells [46,47]. TGF-β is a pleiotropic cytokine that plays a central role in maintaining epithelial homeostasis. In early carcinogenesis, TGF-β acts as a tumor suppressor by inhibiting cell proliferation [49,50]. However, several studies showed that primary tumor cells in the late stage could reprogram their response to TGF-β by dysregulation or mutational inactivation of various components of the TGF-β signaling pathway and through cross-interaction with other oncogenic pathways. Consequently, the TGF-β signal becomes a bone metastasis-promoting one [51–53]. Blocking TGF-β signaling especially in advanced stages of cancer may result in beneficial therapeutic responses by inhibiting metastatic progression [54]. TGF-β is one of the most potent inducers of CCN2, promoting CCN2 expression in bone metastatic cancer cells [39]; and the induction occurs through a complex network of transcriptional interactions requiring Smads, protein kinase C, and ras/MEK/ERK, as well as an Ets-1/ transcription enhancer factor binding element in the CCN2 promoter [55–57].
3.3. Bone marrow stromal and osteoblastic cells-produced RANKL

The RANK/RANKL signaling pathway has been identified as the key molecular basis for osteoclast-mediated bone resorption in both normal bone remodeling and in pathological conditions, including bone metastasis [58–60]. RANK is a transmembrane signaling receptor of the tumor necrosis factor (TNF) receptor superfamily that is expressed on the surface of osteoclast precursors [61,62]. Its cognate ligand, RANKL, is expressed almost exclusively within the bone marrow stromal cell compartment and is up-regulated by most hormones and factors that stimulate bone resorption [7,60]. The interaction of RANK and RANKL is necessary for osteoclast formation, function, and survival [58,63]. RANKL (50 ng/ml) stimulates osteoclastogenesis in mouse total bone

Figure 2  Radiographic and immunohistochemical analysis of bone from mice bearing MDA-MB-231 or oral squamous cell carcinoma of the mandibular region. (A) Representative radiographs of hind limbs from 25 days after tumor inoculation and treated with control IgG or neutralizing CCN2 antibody (CCN2 AB). The arrowheads indicate osteolytic lesions. (B) Localization patterns of CCN2, PTHrP, or PTH1R. Scale bar, 50 μm. (C) Representative radiograph of invasive oral squamous cell carcinoma in the mandibular region. (D) Immunohistochemical staining of CCN2 in section of invasive pattern of resected mandible. Scale bar 200 μm. The data were modified from Shimo et al. [45] (A and B) and Shimo et al. [33] (C and D). Bn: bone; Tm: tumor.
marrow cells in the presence of 100 ng/ml CCN2 (Fig. 2D) [33]. Stromal/osteoblastic cells are essential for in vitro osteoclastogenesis through cell-to-cell interactions [64]. Therefore, it has been hypothesized that CCN2 may facilitate cell-to-cell signaling by interacting with multiple molecules on the surface of these cells through integrin [19,65], proteoglycans [66], and growth factors [18].

3.4. Tumor-produced endothelin-1 (ET-1)

Tumor-produced endothelin-1 (ET-1) is also a key mediator of osteoblastic bone metastasis, which is characteristic of breast and prostate cancers [67,68]. CCN2 is one of the secreted factors downstream of ET-1, as determined from microarray analysis of osteoblasts [69]. ET-1 activates the CCN2 promoter and induces CCN2 expression in cardiomyocyte cells [70]. Furthermore, ET-1 induces CCN2 in an additive fashion to TGF-β through an element distinct from the TGF-β response element [71—73].

4. General role and mechanism of CCN2 in bone microenvironment

In the bone marrow microenvironment affected by tumor, substantial bone marrow angiogenesis is present compared with healthy persons [74]. In the case of the best-characterized CCN2, this factor is known to promote the proliferation and differentiation of vascular endothelial cells as well as fibroblasts and osteoblasts [22,24,25,75]. CCN2 protein is able to interact with multiple molecules in the bone microenvironment, thus resulting in the modulation of the extra cellular molecular network therein. The angiogenic effects of CCN2 is the results of the interaction with adhesion molecules [19], cell-surface signal transducing receptors [76], proteoglycans [66] and growth factors [18].

Bone-derived growth factors, such as TGF-β, FGFs, PDGFs, BMPs, and IGF-1 are activated and released into the bone microenvironment. Elevated TGF-β does not appear to affect tumor growth, but rather leads to the production of PTHrP [77] and CCN2 [39,45] in breast cancer cells, thus establishing a continuously destructive cycle termed the "vicious cycle" through up-regulation of RANKL and accelerated bone resorption. Of note, CCN2 is known to interact with these growth factors [16,18] or regulate the gene expression of some of them [37]. As a result, CCN2 may be anticipated to modulate the effects of these growth factors toward the osteoblast induced RANKL and OPG expression, osteoclast formation or osteoclast activation in bone metastasis region (Fig. 3). The other critical function of CCN2 is exerted under the interaction with extracellular matrix (ECM) molecules and cell adhesion molecules. By interacting with integrins, functions and other proteins and proteoglycans, CCN2 may promote adhesion and migration of osteoclast precursor cells and stimulate osteoclast formation and activation (Fig. 3). CCN2 may be an integrator/modulator of osteoclast precursor and bone destruction within the skeleton [78—80] (Figs. 3 and 4).

5. Therapeutic approaches

Suppression of bone-lesion development and limiting the progression of an established bone metastasis should be the primary goals of treating metastatic bone disease. Osteoclastic activity is the major contributor to cancer-induced bone disease, and this cell type is an ideal target for therapies. For instance, bisphosphonates have been widely and successfully used for the treatment of bone metastases in breast cancer and multiple myeloma patients [81—84], as they block not only bone resorption but also tumor-cell mitosis and additionally stimulate tumor-cell apoptosis [85].

5.1. OPG

The OPG/RANK/RANKL pathway offers multiple molecular checkpoints for therapeutic targeting of osteolytic metastases [86,87]. In a randomized, double-blind, phase-I clinical trial, a single subcutaneous dose of the recombinant OPG construct AMGN-0007 was effective in suppressing bone resorption in breast cancer patients with established skeletal metastasis [88]. Although AMGN-0007 had favorable pharmacokinetic and pharmacodynamic properties in the cancer patient population, one potential risk with a recombinant OPG molecule would be the generation of antibody titers against the endogenous OPG protein.
5.2. RANKL

RANKL inhibitor, denosumab (formerly AMG 162), has also been developed and is being tested in the clinic. Denosumab is a fully human monoclonal antibody against RANKL. Phase-I and -II clinical trials have shown that denosumab suppressed bone resorption in patients with malignant bone disease stemming from multiple myeloma, prostate, or breast cancer [89–91]. Denosumab was generally well tolerated in those trials. No related serious adverse events occurred. Furthermore, no patient had detectable anti-denosumab antibodies. Another method to target RANKL includes an osteoprotegerin-like peptidomimetic (OP3-4), which was demonstrated to be capable of inhibiting myeloma bone disease in vivo model [92]. No clinical results using these latter strategies have been presented to date.

5.3. PTHrP

PTHrP antibodies neutralize PTHrP and vitamin-D analogues and decrease PTHrP production [93]. PTHrP neutralizing antibodies are therapeutically effective in animal models of the humoral hypercalcemia of malignancy and those of bone metastasis [94,95].

5.4. Src

Because of the role of Src in both cancer development and in bone metabolism, it may provide a therapeutic target for patients with bone metastases. Dasatinib (SPRYCEL, Bristol-Myers Squibb, New York) is a small-molecule tyrosine kinase inhibitor with activity against several signaling proteins [96]. Dasatinib is now being evaluated in phase-I and II trials in a variety of tumor types, including prostate and lung cancers [97,98]. AZD-0530 is an another dual Src/Abl inhibitor that has been shown to inhibit the formation and activity of human osteoclasts, as well as to suppress tumor growth and metastasis [89].

5.5. ET-1

Specific inhibitors of ET-1 signaling (antagonists of the endothelin-A receptor [ETAR]) have beneficial effects on bone metastatic lesions arising from prostate cancer [99]. Selective ETAR antagonists may block the proliferative effects of exogenous ET-1 on both prostate cancer cells and osteoblasts. More specifically, Atrasentan (ABT-627), an antagonist of ETARs, may inhibit tumor growth in bone both by direct effects on the tumor cells and by destroying important bone/tumor interactions [100–102]. ZD4054, a specific ETA antagonist, is
being examined currently in phase-II studies on prostate cancer, little data on its efficacy has been reported, but an improvement was seen in overall survival [103].

5.6. Radionuclide

Radionuclide therapy is a useful and cost-effective means of alleviating bone pain in metastatic disease and may be more effective when combined with chemotherapy, bisphosphonates, and radiation therapy [104,105]. Due to the chemical similarity between strontium and calcium, $^{89}$Sr (Metastron$^{10}$) is preferentially retained in the skeleton, especially in areas of rapid osteoblastic activity and bone formation associated with tumor-mediated bone remodeling by a factor of about 10 versus its retention in healthy bone [104]. $^{153}$Sm-EDTMP (Quadramet$^{10}$) is most widely used in the United States to relieve pain from bone metastasis, with palliation occurring in 65–80% of patients with better overall response rates at higher doses in early phase-I and -II studies [105]. $^{153}$Sm is administered with a large excess of a bone targeting phosphate-chelating agent [lexidronam or ethylenediaminetetramethylenephosphonate (EDTMAP)] to enable delivery of the injected $^{153}$Sm to areas of bone formation. The absorption of this radiopharmaceutical is 17 times faster in lesions than in normal bone, and due to its rapid renal clearance, nonosseous radioactive exposure is low [106].

6. Conclusions

Osteoclastogenesis and angiogenesis is the most fundamental step leading to tumor-induced bone destruction. Based on the accumulating data in vitro and the phenotypic change observed in the CCN2 KO mice, CCN2 is now believed to be such a central modulator of extracellular signaling and molecular architecture in the endochondral ossification process. Final stage of endochondral ossification is the most fundamental step for osteoclast activation and angiogenesis from bone marrow. From a clinical point or view, osteoclast and angiogenesis is one of the major targets of tumor bone metastasis (Fig. 4). Unlike tumor growth, the major modulator of this process has been shown to be the CCN2 molecule, which is thus now regarded as a potential target of anti-osteoclastogenic and angiogenic therapy [45,107]. In fact, recent studies have shown that CCN2 stimulated TRAP-positive osteoclast-like cell formation in bone marrow cell culture [33]. Therefore, an anti-CCN2 strategy may provide a safer choice without adverse effects than those with other osteoclast targeted agents. CCN2 may be able to apply for molecular target therapy of bone metabolic and resorptive disease such as osteoporosis and periodontitis.

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References


Role of CCN2 in bone microenvironment


