We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

6,600
Open access books available

177,000
International authors and editors

195M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Role of Connective Tissue Growth Factor (CTGF/CCN2) in Oral Squamous Cell Carcinoma-Induced Bone Destruction

Tsuyoshi Shimo* and Akira Sasaki
Department of Oral and Maxillofacial Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama Japan

1. Introduction

Oral squamous cell carcinoma cells in the gingiva frequently invade the maxillary or mandibular bone. The clinical consequences of oral squamous cell carcinoma-induced bone destruction include a worse prognosis, a high morbidity rate, hypercalcemia, and nerve paralysis (Brown, et al., 2002; Hicks, et al., 1997; Shaw, et al., 2004). Patients with oral squamous cell carcinoma and associated bone invasion require bone resection, which has a major influence on their functional outcome. However, the mechanism of bone destruction by oral squamous cell carcinoma remains unresolved.

Localization of tumor cells within the bone leads to the production of tumor-associated factors synthesized either 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 β ligand (RANKL) and/or a decrease in that of osteoprotegerin (OPG), which ultimately results in the activation and survival of osteoclasts, with osteolytic lesions being the result (Roodman GD & Dougall WC, 2008). Bone destruction 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; (Kayamori, et al., 2010; Roodman GD, 2004; Roodman GD & Dougall WC, 2008; Shibahara, et al., 2005). 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 vicious cycle continues.

Connective tissue growth factor (CTGF/CCN2) is a member of the CCN family (Takigawa M, et al., 2003), which consists of 6 members: CCN1 (Cyr61), CCN2 (CTGF), CCN3 (NOV), CCN4 (WISP-1), CCN5 (WISP-2), and CCN6 (WISP-3; (Katsube K, et al., 2009; Kubota S & Takigawa M, 2007b; Perbal B, 2004), all of which possess an NH2-terminal signal peptide

*Corresponding Author

www.intechopen.com
indicative of their secreted-protein nature. CCN proteins share a common molecular structure consisting of an 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 in the case of CCN5, which lacks the CT module. The N-terminal and C-terminal halves of the proteins are connected by a hinge region that is not conserved and is particularly sensitive to proteolysis (Dean, et al., 2007; Kireeva, et al., 1996). By means of these modules, the CCN2 protein interacts with a number of extracellular molecules. The IGFBP motif is responsible for binding IGF (Bork P, 1993), albeit studies with CCN2 have demonstrated that the interaction of CCN2 with IGF occurs with a much lower affinity than that of authentic IGFBPs (Yang DH, et al., 1998 Jul). The VWC motif binds to integrin αvβ3 (Perbal B & Takigawa M, 2005) and has been implicated as a binding site for BMP-4 and TGF-β family members, this binding modulating their activity (Abreu JG, et al., 2002). The TSP-1 motif is involved in binding to integrin α6β1, αvβ3 (Perbal B & Takigawa M, 2005), LRPI and LRPs (Gao & Brigstock, 2003; Segarini PR, et al., 2001), and VEGF (Inoki I, et al., 2002). Finally, the CT motif binds integrin αvβ3 and cell-surface heparan sulfate proteoglycans (HSPGs; Gao R & Brigstock DR, 2004). These different domains of CCN2 could be responsible for the differential signaling resulting in its various biological activities (Fig. 1).

Fig. 1. CCN2-interacting proteins and receptors of CCN2. CCN2 protein interacts with a variety of cell-surface signal-transducing receptors and extracellular ligands, including various integrins, heparan sulfate proteoglycans (HSPG), and LRPs. Receptors and extracellular proteins that interact with 3 of the 4 conserved CCN2 domains are shown.

One of the most prominent functions of CCN2 is its role in cell adhesion. When immobilized on solid surfaces in cell cultures, CCN2 proteins can support the adhesion of most adherent-cell types through integrins and HSPGs and induce adhesive signaling. Adhesion of CCN2 to human skin fibroblasts occurs through α6β1-HSPGs and rapidly induces the formation of α6β1-containing focal adhesion complexes, activation of focal adhesion kinase (FAK), paxillin, and Rac, as well as reorganization of the actin cytoskeleton and formation of filopodia and lamellipodia (C. C. Chen, et al., 2001). CCN2 can serve as an adaptor for other extracellular matrix proteins to promote cell adhesion, as exemplified by the binding of CCN2 to fibronectin and perlecan (Y. Chen, et al., 2004; Nishida, et al., 2003). In addition to supporting cell adhesion, one of the ubiquitous activities of CCN proteins is the regulation of cell migration.
CCN2 proteins stimulate the migration of many mesenchymal cell types (Babic, et al., 1999; Grzeszkiewicz, et al., 2001; Lin, et al., 2003; Shimo T, et al., 1998; Shimo T, et al., 1999).

CCN2 was originally discovered in, and purified from, the conditioned medium of cultured vein endothelial cells (Bradham, et al., 1991). In 1998, Shimo et al. reported that knockdown of ccn2 expression results in the suppression of the proliferation and migration of normal vascular endothelial cells (Shimo T, et al., 1998). Subsequently, CCN2 was shown to induce angiogenesis in corneal implants (Babic, et al., 1999) and chick chorioallatonic membranes (Shimo T, et al., 1999). CCN2 also induces chemotaxis (inducing directional cell migration) and chemokinesis (random cell movement) in endothelial cells (Babic, et al., 1999; Babic, et al., 1998; Lin, et al., 2003; Shimo T, et al., 1999). Through direct binding to integrin αvβ3, CCN2 can recapitulate angiogenic events in vitro by promoting endothelial cell adhesion, migration, proliferation, and tube formation (Babic, et al., 1999; Leu, et al., 2002; Lin, et al., 2003; Shimo T, et al., 1999).

CCN2 knockout mice die just after birth due to respiratory failure (Ivkovic S, et al., 2003). This failure is attributed to hypoplasia of the thoracic skeleton and deformity of the oral cavity (palatal cleft and shortened mandible). CCN2 knockout mice also show skeletal dysmorphisms as a result of impaired chondrocyte proliferation and reduced extracellular matrix with altered 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 enchondral ossification (Nakanishi T, et al., 2000; Nishida T, et al., 2000; Shimo T, et al., 2005). 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. The biological activities of CCN2 also include the development of Meckel’s cartilage (Shimo T, et al., 2004) and tooth germs (Shimo T, et al., 2002).

Next we will summarize research indicating the essential roles of CCN2 and related molecules in the bone destruction caused by cancer.

2. Cancers and CCN2

CCN2 proteins carry out their biological activity through binding and cell surface integrins (Lau LF & Lam SC, 1999), and elevated CCN2 expression has been observed in breast cancers (Xie D, et al., 2001), pancreatic cancers (Wenger C, et al., 1999), melanomas (Kubo M, et al., 1998), chondrosarcomas (Shakunaga T, et al., 2000), and squamous cell carcinomas (Shimo T, et al., 2008). Although CCN2 shows multiple roles in various cancer types, in breast tumor cells CCN2 over-expression has been linked to an increase in tumor size, lymph node metastasis (Chen PS, et al., 2007; Xie D, et al., 2001), and drug resistance through up-regulation of the survival pathway (Wang MY, et al., 2009). CCN2 is also regarded as a central mediator of tumor angiogenic factor in certain malignancies (Kondo S, et al., 2002; Shimo T, et al., 2001a; Shimo T, et al., 2001b). 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 collaboration with other factors (Kang Y, et al., 2003; Minn AJ, et al., 2005). Neutralizing antibodies against CCN2 significantly inhibit local tumor growth,
angiogenesis, and osteolysis caused by metastatic human breast cancer cells (Shimo T, et al., 2006). CCN2 and PTHrP are strongly expressed in cancer cells that have invaded the bone matrix, and CCN2 expression is regulated by PTHrP through PKA, PKC, and ERK1/2 MAPK pathways (Shimo T, et al., 2006). Furthermore, the CCN2 gene is significantly over-expressed in overt metastatic tumor cells as compared with its expression in disseminated tumor cells in the bone marrow of breast cancer patients by CT-guided bone metastasis biopsy and bone marrow biopsy (Cawthorn, et al., 2009). Fig. 2A illustrates a representative radiographic pattern of invasive bone destruction observed in a patient with oral squamous cell carcinoma in the mandibular region. In such cases, as shown in Fig. 2B, tumor cells fill the bone marrow space and destroy both the trabecular and cortical bone of the mandible.

Fig. 2. Radiographic and immunohistochemical analysis of oral squamous cell carcinoma of the mandibular region. (A) Representative radiograph of an invasive oral squamous cell carcinoma in the mandibular region (arrowheads). (B) HE-stained sections of the resected mandible. Tumor tissue (Tm) has invaded into the marrow cavity and replaced the normal cellular elements. Significant loss of trabecular and cortical bone (Bn) has occurred. (C and D) Immunohistochemical staining of CCN2 in a section of invasive tumor (C) and of osteoclasts (D, arrowheads) of the resected mandible. Scale bar = 100 µm. Bn: Bone, Tm: Tumor. The data were modified from Shimo et al. (Shimo, et al., 2008) (B and D).
CCN2 is abundantly produced by the tumor cells that have invaded the bone matrix (Fig. 2C); and, interestingly, CCN2 is also present in the osteoclasts at the destroyed bone/tumor cell interface (Fig. 2D, arrowheads). Of note, up-regulation of CCN2 in oral squamous cell carcinoma of the mandible is associated with increased bone destruction (Shimo T, et al., 2008). These data suggest that CCN2 can be considered both a diagnostic marker and target for treatment of oral osteolytic mandibular squamous cell carcinoma.

3. Relation between CCN2 and tumor associated factors and signaling

3.1 Insulin-like growth factor (IGF) and CCN2

The insulin-like growth factor (IGF) is the most abundant factor stored in the bone matrix (Hauschka, et al., 1986). The IGF system comprises hormone-like growth factors (IGF-I and II), cell-surface receptors (IGF-IR, IGF-IIR and insulin receptor), circulating binding proteins (IGFBPs), and IGFBP proteases. Activation of IGF-I/IGFR signaling plays an important role in cancer cells, leading to an increase in cell proliferation, invasion/migration, to a decrease in apoptosis, and to resistance to antineoplastic agents, suggesting that IGF/IGFR plays an important role in mammary tumorigenesis (Brady, et al., 2007; Kimura, et al., 2010; Saxena, et al., 2008). A larger family of secreted cysteine-rich proteins, made so by inclusion of the Twisted gastrulation (TSG), IGFBP, and CCN families, is termed TIC (Flint, et al., 2008; Pell, et al., 2005). Interestingly, members of the CCN protein family bind IGF with low affinity (Hwa, et al., 1999). However, there are only a few published reports on the association between IGF system and CCN proteins.

3.2 Parathyroid hormone-related protein (PTHrP) and CCN2

Parathyroid hormone-related protein (PTHrP) has important developmental roles in the embryonic skeleton and other tissues. Detection or increased plasma concentrations of PTHrP have been found in 80% of hypercalcemia patients with solid tumor (Burtis WJ, et al., 1990). When it is produced in excess by cancer cells, 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 (Guise TA, et al., 1996). PTHrP induces both the production of RANKL and down-regulation of OPG production by osteoblasts, thereby stimulating osteoclastogenesis (Horwood NJ, et al., 1998; Lee SK & Lorenzo JA, 1999). Oral squamous cell carcinoma cells provide a suitable microenvironment for osteoclast formation by producing PTHrP (Kawamori, et al., 2010). Knock-down of PTHrP in oral squamous cell carcinoma cell caused decreased osteoclast formation in vitro, and suppressed tumor bone invasion in vivo (Y. Takayama, et al., 2010). Sections of resected mandibles from patients with invasive oral squamous cell carcinoma showed strong expression of PTHrP in tumor cells and great number of osteoclasts at bone invasion sites (Y. Takayama, et al., 2010). Type I PTH/PTHrP receptor (PTH1R) expression is specifically observed in cancer cells producing PTHrP and CCN2 that have invaded the bone marrow, and PTHrP strongly up-regulates CCN2 in MDA-MB-231 cells in vitro (Shimo T, et al., 2006). CCN2 is also critically involved in osteolytic metastasis and is induced by PKA- and PKC-dependent activation of ERK 1/2 signaling by PTHrP (Shimo T, et al., 2006).
3.3 Transforming growth factor-β (TGF-β) and CCN2

TGF-β is by far the second abundant cytokine in bone, and must be considered as a central player in bone turnover (Bonewald LF & Mundy GR, 1990) and potentially able to couple bone resorption with bone formation (Karsdal MA, et al., 2001; Takeshita S, et al., 2000). Restricted to the bone environment, target cells of TGF-β include cancer cells as well as osteoblasts, osteoclasts, their precursors in the bone marrow, and stromal cells (Bonewald LF & Mundy GR, 1990; Karsdal MA, et al., 2001). 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 (Massague J, et al., 2000; Sun L, 2004). However, several studies showed that primary tumor cells in the late stage can 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 (Nagaraj & Datta, 2010).

TGF-β transduces its signal through 2 highly conserved single transmembrane serine/threonine kinase receptors, termed type I (TβRI) and type II (TβRII). TβRII activates TβRI upon formation of a ligand–receptor complex by hyperphosphorylating serine/threonine residues in the CS region of TβRI. Activated TβRI in turn phosphorylates Smad2 and Smad3, which interact with Smad4. Their complex is translocated to the nucleus, where it regulates the transcription of target genes. This signaling cascade initiates broad cellular and noncellular processes including proliferation and differentiation, migration and motility, and deposition of extracellular matrix, as well as induces the production of cytokines contributing to tumorigenesis, metastasis, and angiogenesis (Ge, et al., 2006; Petersen, et al., 2010). Due to its central role in TGF-β signaling, TβRI is emerging as a novel target for the blockade of the tumor-promoting and metastasis activities of the TGF-β pathway (Shinto, et al., 2010). Consequently, the TGF-β signal becomes a bone metastasis-promoting one (Kang Y, et al., 2005; Kominsky SL, et al., 2007; Yin JJ, et al., 1999). TβRI-positive signals are closely associated with destructive invasion of the mandible by oral squamous cell carcinoma cells, and a TβRI-inhibitor greatly reduces oral squamous cell carcinoma cell-induced bone destruction and osteoclast formation both in vivo and in vitro (Goda, et al., 2010).

TGF-β is one of the most potent inducers of CCN2, promoting CCN2 expression in bone metastatic cancer cells (Kang Y, et al., 2003); 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 (Chen Y, et al., 2002; Leask A, et al., 2002; Van Beek JP, et al., 2006). TGF-β released from the bone causes a further increase in the expression of the TGF-β-responsive osteoclast-inducing genes, CCN2, RANKL, and TNF-α in oral squamous cell carcinoma cells, thus establishing a composite positive-feedback cycle of metastasis (Kang Y, et al., 2003; Shimo T, et al., 2006).

3.4 RANKL and CCN2

The RANK and its ligand RANKL signaling pathway play pivotal role in osteoclast-mediated bone resorption in both normal bone remodeling and in pathological conditions, including bone metastasis (Boyle WJ, et al., 2003; Lacey DL, et al., 1998; Simonet WS, et al., 1997). RANK is a transmembrane signaling receptor of the tumor necrosis factor (TNF)
Role of Connective Tissue Growth Factor (CTGF/CCN2) in Oral Squamous Cell Carcinoma-Induced Bone Destruction

181

receptor superfamily that is expressed on the surface of osteoclast precursors (Hsu H, et al., 1999; Nakagawa N, et al., 1998). 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 (Boyle WJ, et al., 2003; Roodman GD & Dougall WC, 2008). The interaction between RANK and RANKL is necessary for osteoclast formation, function, and survival (Kong YY, et al., 1999; Lacey DL, et al., 1998). RANKL (50 ng/ml) stimulates osteoclastogenesis in mouse total bone marrow cells in the presence of 100 ng/ml CCN2 (Shimo T, et al., 2008). Stromal/osteoblastic cells are essential for in vitro osteoclastogenesis through cell-to-cell interactions (Kondo Y, et al., 2001). The expression of CCN2 is up-regulated in the cells of mouse macrophage cell line RAW264.7 after treatment with RANKL, and CCN2 synergistically promotes RANKL-induced osteoclast differentiation by interacting with dendritic cell-specific transmembrane protein (DC-STAMP) on the surface of osteoclast-like cells (Nishida, et al., 2011). 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 (Gao R & Brigstock DR, 2004; Hoshijima, et al., 2006), proteoglycans (Nishida, et al., 2003), and growth factors (Inoki I, et al., 2002).

3.5 Endothelin-1 (ET-1) and CCN2

Endothelin-1 (ET-1) is also a key mediator of osteoblastic bone metastasis, which is characteristic of breast and prostate cancers (Nelson JB, et al., 1995; Yin JJ, et al., 2003). Functional inhibition of ET-1 activity by blocking its receptor, ET\(\alpha\), significantly decreases bone metastasis in an experimental bone metastasis model involving the osteoblastic breast cancer cell line ZR-75-1 (Guise, et al., 2003; Yin JJ, et al., 2003).

CCN2 is one of the secreted factors downstream of ET-1, as determined from microarray analysis of osteoblasts (Clines GA, et al., 2007). ET-1 activates the CCN2 promoter and induces CCN2 expression in cardiomyocyte cells (Recchia AG, et al., 2009). Furthermore, ET-1 induces CCN2 in an additive fashion with TGF-\(\beta\) through an element distinct from the TGF-\(\beta\) response element (Horstmeyer A, et al., 2005; Shi-Wen X, et al., 2008; Xu SW, et al., 2004).

3.6 Integrins and CCN2

Integrin have been shown to be critical in controlling how tumor cells interact with their microenvironment. The integrin \(\alpha_\beta_3\) is a receptor for osteopontin, fibronectin, and vitronectin, which are extracellular matrix proteins important in the bone matrix (Schneider, et al., 2011); and \(\alpha_\beta_3\) has been identified as one of the CCN2 receptors (C. C. Chen, et al., 2001). Bone metastatic cancer cells have a higher expression of \(\alpha_\beta_3\) than their primary tumor (Liapis, et al., 1996), promoting adherence to the bone matrix (S. Takayama, et al., 2005). The over-expression of \(\alpha_\beta_3\) in the tumor cells not only leads to increased tumor cell adhesion, and invasion to bone, but also increases osteoclast recruitment at the tumor and bone interface (Pecheur, et al., 2002; Sloan, et al., 2006). Whereas, \(\alpha_\beta_1\), another signaling receptor mediating CCN2 action, plays a necessary role in the binding of prostate cancer tumor cells to the bone stroma (Van der

www.intechopen.com
Bone-invasive destructive tumor cells enhance osteoclast function and recruitment. \( \alpha_\beta_3 \) is the predominant integrin found on osteoclasts, and is responsible for mediating osteoclast-bone recognition (Crippes, et al., 1996; Liapis, et al., 1996; Ross, et al., 1993; Zambonin Zallone, et al., 1989) and subsequent attachment to the bone matrix (Chellaiah, 2006; Ross, et al., 1993). This signaling creates the characteristic resorptive ruffled membrane, as well as regulates OC spreading and the overall organization of the cytoskeleton (Faccio, et al., 2003; McHugh, et al., 2000).

### 3.7 Wnt signaling and CCN2

The Wnt signaling pathways are initiated by a combination of ligands and receptors formed from among 19 secreted Wnt ligands, 10 Frizzled receptors, with the involvement of the coreceptor LRP5/6, which is lipoprotein receptor-related protein 5/6. These ligand-receptor interactions then lead to the activation of multiple intermediate Wnt effectors including \( \beta \)-catenin, c-Jun-NH\( _2 \)-kinase, and calcium-channel regulators. The accumulation of \( \beta \)-catenin in the cytoplasm and its translocation to the nucleus represent the hallmark of the activated canonical Wnt pathway. In the nucleus, \( \beta \)-catenin forms a complex with lymphocyte enhancer factor/T-cell factor family of transcription factors to activate many oncogenes, such as c-Myc, cyclin D1, metalloproteinases, c-Met, etc (Fuerer, et al., 2008; Rubin, et al., 2010).

The Wnt/\( \beta \)-catenin signaling pathway is an important target for eliminating cancer stem cell in head and neck squamous cell carcinomas (Song, et al., 2010). The importance of paracrine Wnt signaling in bone metastasis was first revealed in multiple myeloma (Tian, et al., 2003), a plasma cell leukemia that causes severe osteolytic bone disease. The results revealed that one of the tumor-secreted factors responsible for the enhanced osteolysis is the Wnt-inhibitor DKK-1 (Tian, et al., 2003). In prostate cancer cells, high DKK-1 expression is correlated with osteolytic disease, consistent with the findings in multiple myeloma; whereas low DKK-1 expression is associated with osteosclerotic bone metastases (Schwaninger, et al., 2007). Functional inhibition of Wnt singling by DKK-1 over-expression in prostate cancer cells favors the formation of osteolytic bone metastases (Hall, et al., 2005).

Si et al. (Si, et al., 2006) observed a significant up-regulation of CCN2 gene expression in mesenchymal stem cells that had been stimulated by Wnt3A. Osteoblasts and stromal cells of transgenic mice that over-express CCN2 display reduced Wnt-\( \beta \)-catenin signaling (Smerdel-Ramoya, et al., 2008). Over-expression of CCN2 in esophageal squamous carcinoma cells results in the accumulation and nuclear translocation of \( \beta \)-catenin leading to activation of TCF-LEF signaling and up-regulation of c-myc and cyclin D1 (Deng, et al., 2007).

### 4. Role of CCN2 in tumor/bone microenvironment

In the bone marrow microenvironment affected by a tumor, substantial bone marrow angiogenesis is present compared with that in healthy persons (Chavez-Macgregor, et al., 2005). CCN2, the best-characterized factor in its family is known to promote the proliferation
Fig. 3. Role of CCN2 in tumor-induced bone destruction. The cross-talk between tumor cells and osteoclasts is not direct, but involves molecular and cellular intermediates; e.g., tumor cells secrete parathyroid-hormone-related peptide (PTHrP), which is the primary stimulator of osteoblast production of RANKL (Roodman GD, 2004). PTHrP induces CCN2 in tumor cells (Shimo T, et al., 2008); on the other hand, PTHrP both up-regulates the production of RANKL and down-regulates OPG production by osteoblasts, thereby stimulating osteoclastogenesis (Horwood NJ, et al., 1998). Other factors produced and secreted by tumor cells (TNF-\(\alpha\), TGF-\(\beta\), macrophage colony-stimulating factor (M-CSF), IL-6, and prostaglandin E2) also increase the expression of RANKL. The increased expression of RANKL in the tumor environment leads to increased formation, activation, and survival of osteoclasts, which cells cooperate with these tumor-induced growth factors (CCN2, TNF-\(\alpha\), TGF-\(\beta\), and M-CSF), resulting in osteolytic lesions. Osteolysis then leads to the release of growth factors derived from bone, including transforming growth factor-\(\beta\) (TGF-\(\beta\)), insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), and bone morphogenetic proteins (BMPs). These factors increase the production of PTHrP and CCN2 or promote tumor growth directly and cause neovascularization. Bone destruction increases local extracellular calcium (Ca\(^{2+}\)) concentrations, which have also been shown to promote tumor growth and the production of PTHrP (Roodman GD, 2004). Thus, tumor cell proliferation and production of tumor-associated factors through the signaling of positive-feedback pathways is promoted, thus giving rise to a “vicious cycle.” The Scheme was modified from (Shimo T, et al., 2011).
and differentiation of not only vascular endothelial cells but also fibroblasts and osteoblasts (C. C. Chen, et al., 2001; Nishida T, et al., 2000; Shimo T, et al., 1998; Shimo T, et al., 1999). CCN2 protein is able to interact with multiple molecules in the bone microenvironment, which interaction results in the modulation of the extracellular molecular network therein. The angiogenic effect of CCN2 is the result of the interaction of it with adhesion molecules (Gao R & Brigstock DR, 2004), cell-surface signal transducing receptors (Wahab, et al., 2005), proteoglycans (Nishida, et al., 2003), and growth factors (Inoki I, et al., 2002).

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 (Guise TA, 2000) and CCN2 (Kang Y, et al., 2003; Shimo T, et al., 2006) in 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 (Abreu JG, et al., 2002; Inoki I, et al., 2002) or regulate the gene expression of some of them (Shimo T, et al., 2001b). As a result, CCN2 may be anticipated to modulate the effects of these growth factors on the osteoblast-induced RANKL and OPG expression, osteoclast formation or osteoclast activation in regions affected by bone metastasis (Fig. 3). The other critical function of CCN2 is exerted by its interaction with extracellular matrix 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 (Shimo T, et al., 2008). CCN2 not only promotes the expression of DC-STAMP, which plays an important role in cell-cell fusion, but also interacts with this molecule to promote osteoclast differentiation (Nishida, et al., 2011). CCN2 may thus be an integrator/modulator of extracellular information and appears to allow the establishment and progression of tumor angiogenesis and bone destruction within the skeleton (Kubota S & Takigawa M, 2007a; Sasaki A, et al., 1998; Sasaki A, et al., 2003).

5. Conclusions

The initiation of osteoclastogenesis and angiogenesis is the most fundamental step leading to tumor-induced bone destruction. From a clinical point of view, osteoclast formation and angiogenesis would be the major targets of therapeutic drugs for tumor bone metastasis. The major modulator of these processes, referring to osteoclast formation and angiogenesis has been shown to be the CCN2 molecule, which is thus now regarded as a potential target of anti-osteoclastogenic and angiogenic therapy (Aikawa, et al., 2006; Shimo T, et al., 2006). These findings strongly suggest that CCN2 may be a suitable molecular target for therapy of advanced oral squamous cell carcinoma.

6. Acknowledgements

This work was partly supported by grants from the programs Grants-in-Aid for Young Scientists (A) (to T. S.), and Scientific Research (B) (to A. S.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.
7. References

Abreu, J. G., Ketpura, N. I., Reversade, B. & De Robertis, E. M. (2002) Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-beta. Nat Cell Biol, 4, 8, 599-604

Aikawa, T., Gunn, J., Spong, S. M., Klaus, S. J. & Korc, M. (2006) Connective tissue growth factor-specific antibody attenuates tumor growth, metastasis, and angiogenesis in an orthotopic mouse model of pancreatic cancer. Mol Cancer Ther, 5, 5, 1108-1116

Babic, A. M., Kireeva, M. L., Kolesnikova, T. V. & Lau, L. F. (1998) CYR61, a product of a growth factor-inducible immediate early gene, promotes angiogenesis and tumor growth. Proc Natl Acad Sci U S A, 95, 11, 6355-6360

Babic, A. M., Chen, C. C. & Lau, L. F. (1999) Fisp12/mouse connective tissue growth factor mediates endothelial cell adhesion and migration through integrin alphavbeta3, promotes endothelial cell survival, and induces angiogenesis in vivo. Mol Cell Biol, 19, 4, 2958-2966

Bonewald, L. F. & Mundy, G. R. (1990) Role of transforming growth factor-beta in bone remodeling. Clin Orthop Relat Res, 250, 261-276

Bork, P. (1993) The modular architecture of a new family of growth regulators related to connective tissue growth factor. FEBS Lett, 327, 2, 125-130

Boyle, W. J., Simonet, W. S. & Lacey, D. L. (2003) Osteoclast differentiation and activation. Nature, 423, 6937, 337-342

Bradham, D. M., Igarashi, A., Potter, R. L. & Grotendorst, G. R. (1991) Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. J Cell Biol, 114, 6, 1285-1294

Brady, G., Crean, S. J., Naik, P. & Kapas, S. (2007) Upregulation of IGF-2 and IGF-1 receptor expression in oral cancer cell lines. Int J Oncol, 31, 4, 875-881

Brown, J. S., Lowe, D., Kalavrezos, N., D'Souza, J., Magennis, P. & Woolgar, J. (2002) Patterns of invasion and routes of tumor entry into the mandible by oral squamous cell carcinoma. Head Neck, 24, 4, 370-383

Burtis, W. J., Brady, T. G., Orloff, J. J., Erskah, J. B., Warrell, R. P., Jr., Olson, B. R., Wu, T. L., Miltick, M. E., Broadus, A. E. & Stewart, A. F. (1990) Immunochemical characterization of circulating parathyroid hormone-related protein in patients with humoral hypercalcemia of cancer. N Engl J Med, 322, 16, 1106-1112

Cawthorn, T. R., Amir, E., Broom, R., Freedman, O., Gianfelice, D., Barth, D., Wang, D., Holen, I., Done, S. J. & Clemons, M. (2009) Mechanisms and pathways of bone metastasis: challenges and pitfalls of performing molecular research on patient samples. Clin Exp Metastasis, 26, 8, 935-943

Chavez-Macgregor, M., Aviles-Salas, A., Green, D., Fuentes-Alburo, A., Gomez-Ruiz, C. & Aguayo, A. (2005) Angiogenesis in the bone marrow of patients with breast cancer. Clin Cancer Res, 11, 15, 5396-5400

Chellaiah, M. A. (2006) Regulation of podosomes by integrin alphavbeta3 and Rho GTPase-facilitated phosphoinositide signaling. Eur J Cell Biol, 85, 3-4, 311-317

Chen, C. C., Chen, N. & Lau, L. F. (2001) The angiogenic factors Cyr61 and connective tissue growth factor induce adhesive signaling in primary human skin fibroblasts. J Biol Chem, 276, 13, 10443-10452
Chen, P. S., Wang, M. Y., Wu, S. N., Su, J. L., Hong, C. C., Chuang, S. E., Chen, M. W., Hua, K. T., Wu, Y. L., Cha, S. T., Babu, M. S., Chen, C. N., Lee, P. H., Chang, K. J. & Kuo, M. L. (2007) CTGF enhances the motility of breast cancer cells via an integrin-αvβ3-ERK1/2-dependent S100A4-upregulated pathway. *J Cell Sci.*, 120, Pt 12, 2053-2065

Chen, Y., Abraham, D. J., Shi-Wen, X., Pearson, J. D., Black, C. M., Lyons, K. M. & Leask, A. (2004) CCN2 (connective tissue growth factor) promotes fibroblast adhesion to fibronectin. *Mol Biol Cell*, 15, 12, 5635-5646

Chen, Y., Blom, I. E., Sa, S., Goldschmeding, R., Abraham, D. J. & Leask, A. (2002) CTGF expression in mesangial cells: involvement of SMADs, MAP kinase, and PKC. *Kidney Int.*, 62, 4, 1149-1159

Clines, G. A., Mohammad, K. S., Bao, Y., Stephens, O. W., Suva, L. J., Shaughnessy, J. D. Jr., Fox, J. W., Chirgwin, J. M. & Guise, T. A. (2007) Dickkopf homolog 1 mediates endothelin-1-stimulated new bone formation. *Mol Endocrinol.*, 21, 2, 486-498

Crippes, B. A., Engleman, V. W., Settle, S. L., Delarco, J., Ornberg, R. L., Helfrich, M. H., Horton, M. A. & Nickols, G. A. (1996) Antibody to β3 integrin inhibits osteoclast-mediated bone resorption in the thyroparathyroidectomized rat. *Endocrinology*, 137, 3, 918-924

Dean, R. A., Butler, G. S., Hamma-Kourbali, Y., Delbe, J., Brigstock, D. R., Courty, J. & Overall, C. M. (2007) Identification of candidate angiogenic inhibitors processed by matrix metalloproteinase 2 (MMP-2) in cell-based proteomic screens: disruption of vascular endothelial growth factor (VEGF)/heparin affin regulatory peptide (pleiotrophin) and VEGF/Connective tissue growth factor angiogenic inhibitory complexes by MMP-2 proteolysis. *Mol Cell Biol.*, 27, 24, 8454-8465

Deng, Y. Z., Chen, P. P., Wang, Y., Yin, D., Koeffler, H. P., Li, B., Tong, X. J. & Xie, D. (2007) Connective tissue growth factor is overexpressed in esophageal squamous cell carcinoma and promotes tumorigenicity through β-catenin-T-cell factor/Lef signaling. *J Biol Chem.*, 282, 50, 36571-36581

Faccio, R., Takeshita, S., Zallone, A., Ross, F. P. & Teitelbaum, S. L. (2003) c-Fms and the αvβ3 integrin collaborate during osteoclast differentiation. *J Clin Invest.*, 111, 5, 749-758

Flier, C., Nussen, R. & Ten Berge, D. (2008) Wnt signalling in development and disease. Max Delbruck Center for Molecular Medicine meeting on Wnt signaling in Development and Disease. *EMBO Rep.*, 9, 2, 134-138

Gao, R. & Brigstock, D. R. (2003) Low density lipoprotein receptor-related protein (LRP) is a heparin-dependent adhesion receptor for connective tissue growth factor (CTGF) in rat activated hepatic stellate cells. *Hepatol Res.*, 27, 3, 214-220

Gao, R. & Brigstock, D. R. (2004) Connective tissue growth factor (CCN2) induces adhesion of rat activated hepatic stellate cells by binding of its C-terminal domain to integrin αvβ3 and heparan sulfate proteoglycan. *J Biol Chem.*, 279, 10, 8848-8855
Role of Connective Tissue Growth Factor (CTGF/CCN2) in Oral Squamous Cell Carcinoma-Induced Bone Destruction

Ge, R., Rajeev, V., Ray, P., Lattime, E., Rittling, S., Medicherla, S., Protter, A., Murphy, A., Chakravarty, J., Dugar, S., Schreiner, G., Barnard, N. & Reiss, M. (2006) Inhibition of growth and metastasis of mouse mammary carcinoma by selective inhibitor of transforming growth factor-beta type I receptor kinase in vivo. Clin Cancer Res, 12, 14 Pt 1, 4315-4330

Goda, T., Shimo, T., Yoshihama, Y., Hassan, N. M., Ibaragi, S., Kurio, N., Okui, T., Honami, T., Kishimoto, K. & Sasaki, A. (2010) Bone destruction by invading oral squamous carcinoma cells mediated by the transforming growth factor-beta signalling pathway. Anticancer Res, 30, 7, 2615-2623

Grzeszkiewicz, T. M., Kirschling, D. J., Chen, N. & Lau, L. F. (2001) CYR61 stimulates human skin fibroblast migration through Integrin alpha vbeta 5 and enhances mitogenesis through integrin alpha vbeta 3, independent of its carboxyl-terminal domain. J Biol Chem, 276, 24, 21943-21950

Guise, T. A., Yin, J. J. & Mohammad, K. S. (2003) Role of endothelin-1 in osteoblastic bone metastases. Cancer, 97, 3 Suppl, 779-784

Guise, T. A., Yin, J. J., Taylor, S. D., Kumagai, Y., Dallas, M., Boyce, B. F., Yoneda, T. & Mundy, G. R. (1996) Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. J Clin Invest, 98, 7, 1544-1549

Guise, T. A. (2000) Molecular mechanisms of osteolytic bone metastases. Cancer, 88, 12 Suppl, 2892-2898

Hall, C. L., Bafico, A., Dai, J., Aaronson, S. A. & Keller, E. T. (2005) Prostate cancer cells promote osteoblastic bone metastases through Wnts. Cancer Res, 65, 17, 7554-7560

Hauschka, P. V., Mavrakos, A. E., Iafrati, M. D., Doleman, S. E. & Klagsbrun, M. (1986) Growth factors in bone matrix. Isolation of multiple types by affinity chromatography on heparin-Sepharose. J Biol Chem, 261, 27, 12665-12674

Hicks, W. L., Jr., Loree, T. R., Garcia, R. L., Maamoun, S., Marshall, D., Orner, J. B., Bakamjian, V. Y. & Shedd, D. P. (1997) Squamous cell carcinoma of the floor of mouth: a 20-year review. Head Neck, 19, 5, 400-405

Horstmeyer, A., Licht, C., Scherr, G., Eckes, B. & Krieg, T. (2005) Signalling and regulation of collagen I synthesis by ET-1 and TGF-beta1. FEBS J, 272, 24, 6297-6309

Horwood, N. J., Elliott, J., Martin, T. J. & Gillespie, M. T. (1998) Osteotropic agents regulate the expression of osteoclast differentiation factor and osteoprotegerin in osteoblastic stromal cells. Endocrinology, 139, 11, 4743-4746

Hoshijima, M., Hattori, T., Inoue, M., Araki, D., Hanagata, H., Miyauchi, A. & Takigawa, M. (2006) CT domain of CCN2/CTGF directly interacts with fibronectin and enhances cell adhesion of chondrocytes through integrin alpha5beta1. FEBS Lett, 580, 13, 1376-1382

Hsu, H., Lacey, D. L., Dunstan, C. R., Solovyev, I., Colombo, A., Timms, E., Tan, H. L., Elliott, G., Kelley, M. J., Sarosi, I., Wang, L., Xia, X. Z., Elliott, R., Chiu, L., Black, T., Scully, S., Capparelli, C., Morony, S., Shimamato, G., Bass, M. B. & Boyle, W. J. (1999) Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci U S A, 96, 7, 3540-3545
Squamous Cell Carcinoma

Hwa, V., Oh, Y. & Rosenfeld, R. G. (1999) The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr Rev, 20*, 6, 761-787

Inoki, I., Shiomi, T., Hashimoto, G., Enomoto, H., Nakamura, H., Makino, K., Ikeda, E., Takata, S., Kobayashi, K. & Okada, Y. (2002) Connective tissue growth factor binds vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. *FASEB J, 16*, 2, 219-221

Ivkovic, S., Yoon, B. S., Popoff, S. N., Safadi, F. F., Libuda, D. E., Stephenson, R. C., Daluiski, A. & Lyons, K. M. (2003) Connective tissue growth factor coordinates chondrogenesis and angiogenesis during skeletal development. *Development, 130*, 12, 2779-2791

Kang, Y., Siegel, P. M., Shu, W., Drobnjak, M., Kakonen, S. M., Cordon-Cardo, C., Guise, T. A. & Massagué, J. (2003) A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell, 3*, 6, 537-549

Karsdal, M. A., Fjording, M. S, Foged, N. T, Delaissé, J. M & Lochter, A. (2001) Transforming growth factor-beta-induced osteoblast elongation regulates osteoclastic bone resorption through a p38 mitogen-activated protein kinase- and matrix metalloproteinase-dependent pathway. *J Biol Chem, 276*, 42, 39350-39358

Katsube, K., Sakamoto, K., Tamamura, Y. & Yamaguchi, A. (2009) Role of CCN, a vertebrate specific gene family, in development. *Dev Growth Differ, 51*, 1, 55-67

Kayamori, K., Sakamoto, K., Nakashima, T., Takayangi, H., Morita, K., Omura, K., Nguyen, S. T., Miki, Y., Imura, T., Himeno, A., Akashi, T., Yamada-Okabe, H., Ogata, E. & Yamaguchi, A. (2010) Roles of interleukin-6 and parathyroid hormone-related peptide in osteoclast formation associated with oral cancers: significance of interleukin-6 synthesized by stromal cells in response to cancer cells. *Am J Pathol, 176*, 2, 968-980

Kimura, T., Kuwata, T., Ashimine, S., Yamazaki, M., Yamauchi, C., Nagai, K., Ikehara, A., Feng, Y., Dimitrov, D. S., Saito, S. & Ochiai, A. (2010) Targeting of bone-derived insulin-like growth factor-II by a human neutralizing antibody suppresses the growth of prostate cancer cells in a human bone environment. *Clin Cancer Res, 16*, 1, 121-129

Kireeva, M. L., Mo, F. E., Yang, G. P. & Lau, L. F. (1996) Cyr61, a product of a growth factor-inducible immediate-early gene, promotes cell proliferation, migration, and adhesion. *Mol Cell Biol, 16*, 4, 1326-1334

Kondo, S., Kubota, S., Shimo, T., Nishida, T., Yosimichi, G., Eguchi, T., Sugahara, T. & Takigawa, M. (2002) Connective tissue growth factor increased by hypoxia may initiate angiogenesis in collaboration with matrix metalloproteinases. *Carcinogenesis, 23*, 5, 769-776

Kondo, Y., Irie, K., Ikegame, M., Ejiri, S., Hanada, K. & Ozawa, H. (2001) Role of stromal cells in osteoclast differentiation in bone marrow. *J Bone Miner Metab, 19*, 6, 352-358

Kong, Y. Y., Yoshida, H., Sarosi, I., Tan, H. L., Timms, E., Capparelli, C., Morony, S., Oliveira-dos-Santos, A. J., Van, G., Itie, A., Khoo, W., Wakeham, A., Dunstan, C. R., Lacey, D. L., Mak, T. W., Boyle, W. J. & Penninger, J. M. (1999) OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature, 397*, 6717, 315-323
Korah, R., Boots, M. & Wieder, R. (2004) Integrin alpha5beta1 promotes survival of growth-arrested breast cancer cells: an in vitro paradigm for breast cancer dormancy in bone marrow. *Cancer Res*, 64, 13, 4514-4522

Kubo, M., Kikuchi, K., Nashiro, K., Kakinuma, T., Hayashi, N., Nanko, H. & Tamaki, K. (1998) Expression of fibrogenic cytokines in desmoplastic malignant melanoma. *Br J Dermatol*, 139, 2, 192-197

Kubota, S. & Takigawa, M. (2007a) CCN family proteins and angiogenesis: from embryo to adulthood. *Angiogenesis*, 10, 1, 1-11

Kubota, S. & Takigawa, M. (2007b) Role of CCN2/CTGF/Hcs24 in bone growth. *Int Rev Cytol*, 257, 1-41

Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, G., Colombo, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y. X., Kaufman, S., Sarosi, I., Shalhoub, V., Senaldi, G., Guo, J., Delaney, J. & Boyle, W. J. (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell*, 93, 2, 165-176

Lau, L. F. & Lam, S. C. (1999) The CCN family of angiogenic regulators: the integrin connection. *Exp Cell Res*, 248, 1, 44-57

Leask, A., Abraham, D. J., Finlay, D. R., Holmes, A., Pennington, D., Shi-Wen, X., Chen, Y., Venstrom, K., Dou, X., Ponticos, M., Black, C., Bernabeu, C., Jackman, J. K., Findell, P. R. & Connolly, M. K. (2002) Dysregulation of transforming growth factor beta signaling in scleroderma: overexpression of endoglin in cutaneous scleroderma fibroblasts. *Arthritis Rheum*, 46, 7, 1857-1865

Lee, S. K. & Lorenzo, J. A. (1999) Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. *Endocrinology*, 140, 8, 3552-3561

Leu, S. J., Lam, S. C. & Lau, L. F. (2002) Pro-angiogenic activities of CYR61 (CCN1) mediated through integrins alphavbeta3 and alpha6beta1 in human umbilical vein endothelial cells. *J Biol Chem*, 277, 48, 46248-46255

Liapis, H., Flath, A. & Kitazawa, S. (1996) Integrin alpha V beta 3 expression by bone-residing breast cancer metastases. *Diag Mol Pathol*, 5, 2, 127-135

Lin, C. G., Leu, S. J., Chen, N., Tebeau, C. M., Lin, S. X., Yeung, C. Y. & Lau, L. F. (2003) CCN3 (NOV) is a novel angiogenic regulator of the CCN protein family. *J Biol Chem*, 278, 26, 24200-24208

Massague, J., Blain, S. W. & Lo, R. S. (2000) TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell*, 103, 2, 295-309

McHugh, K. P., Hodivala-Dilke, K., Zheng, M. H., Namba, N., Lam, J., Novack, D., Feng, X., Ross, F. P., Hynes, R. O. & Teitelbaum, S. L. (2000) Mice lacking beta3 integrins are osteosclerotic because of dysfunctional osteoclasts. *J Clin Invest*, 105, 4, 433-440

Minn, A. J., Kang, Y., Serenganova, I., Gupta, G. P., Giri, D. D., Doubrovin, M., Ponomarev, V., Gerald, W. L., Blasberg, R. & Massagué, J. (2005) Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. *J Clin Invest*, 115, 1, 44-55
Nagaraj, N. S. & Datta, P. K. (2010) Targeting the transforming growth factor-beta signaling pathway in human cancer. Expert Opin Investig Drugs, 19, 1, 77-91

Nakagawa, N., Kinosaki, M., Yamaguchi, K., Shima, N., Yasuda, H., Yano, K., Morinaga, T. & Higashio, K. (1998) RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem Biophys Res Commun, 253, 2, 395-400

Nakanishi, T., Nishida, T., Shimo, T., Kobayashi, K., Kubo, T., Tamatani, T., Tezuka, K. & Takigawa, M. (2000) Effects of CTGF/Hcs24, a product of a hypertrophic chondrocyte-specific gene, on the proliferation and differentiation of chondrocytes in culture. Endocrinology, 141, 1, 264-273

Nelson, J. B., Hedican, S. P., George, D. J., Reddi, A. H., Plantadosi, S., Eisenberger, M. A. & Simons, J. W. (1995) Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nat Med, 1, 9, 944-949

Nishida, T., Kubota, S., Fukunaga, T., Kondo, S., Yosimichi, G., Nakanishi, T., Takano-Yamamoto, T. & Takigawa, M. (2003) CTGF/Hcs24, hypertrophic chondrocyte-specific gene product, interacts with perlecan in regulating the proliferation and differentiation of chondrocytes. J Cell Physiol, 196, 2, 265-275

Nishida, T., Emura, K., Kubota, S., Lyons, K. M. & Takigawa, M. (2011) CCN family 2/connective tissue growth factor (CCN2/CTGF) promotes osteoclastogenesis via induction of and interaction with dendritic cell-specific transmembrane protein (DC-STAMP). J Bone Miner Res, 26, 2, 351-363

Nishida, T., Nakanishi, T., Asano, M., Shimo, T. & Takigawa, M. (2000) Effects of CTGF/Hcs24, a hypertrophic chondrocyte-specific gene product, on the proliferation and differentiation of osteoblastic cells in vitro. J Cell Physiol, 184, 2, 197-206

Pecheur, I., Peyruchaud, O., Serre, C. M., Guglielmi, J., Voland, C., Bourre, F., Margue, C., Cohen-Solal, M., Buffet, A., Kieffer, N. & Clezardin, P. (2002) Integrin alpha(v)beta3 expression confers on tumor cells a greater propensity to metastasize to bone. FASEB J, 16, 10, 1266-1268

Pell, J. M., Salih, D. A., Cobb, L. J., Tripathi, G. & Drozd, A. (2005) The role of insulin-like growth factor binding proteins in development. Rev Endocr Metab Disord, 6, 3, 189-198

Perbal, B. (2004) CCN proteins: multifunctional signalling regulators. Lancet, 363, 9402, 62-64

Perbal, B. & Takigawa, M. (2005) CCN Proteins a New Family of Cell Growthad Differentiation Regulators. Imperial College Press, London,

Petersen, M., Pardali, E., van der Horst, G., Cheung, H., van den Hoogen, C., van der Pluijm, G. & Ten Dijke, P. (2010) Smad2 and Smad3 have opposing roles in breast cancer bone metastasis by differentially affecting tumor angiogenesis. Oncogene, 29, 9, 1351-1361

Recchia, A. G., Filice, E., Pellegrino, D., Dobrini, A., Cerra, M. C. & Maggiolini, M. (2009) Endothelin-1 induces connective tissue growth factor expression in cardiomyocytes. J Mol Cell Cardiol, 46, 3, 352-359

Roodman, G. D. (2004) Mechanisms of bone metastasis. N Engl J Med, 350, 16, 1655-1664

www.intechopen.com
Roodman, G. D. & Dougall, W. C. (2008) RANK ligand as a therapeutic target for bone metastases and multiple myeloma. *Cancer Treat Rev*, 34, 1, 92-101

Ross, F. P., Chappel, J., Alvarez, J. I., Sander, D., Butler, W. T., Farach-Carson, M. C., Mintz, K. A., Robey, P. G., Teitelbaum, S. L. & Cheresh, D. A. (1993) Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin alpha v beta 3 potentiate bone resorption. *J Biol Chem*, 268, 13, 9901-9907

Rubin, E. M., Guo, Y., Tu, K., Xie, J., Zi, X. & Hoang, B. H. (2010) Wnt inhibitory factor 1 decreases tumorigenesis and metastasis in osteosarcoma. *Mol Cancer Ther*, 9, 3, 731-741

Sasaki, A., Alcalde, R. E., Nishiyama, A., Lim, D. D., Mese, H., Akedo, H. & Matsumura, T. (1998) Angiogenesis inhibitor TNP-470 inhibits human breast cancer osteolytic bone metastasis in nude mice through the reduction of bone resorption. *Cancer Res*, 58, 3, 462-467

Sasaki, A., Yoshioka, N. & Shimo, T. (2003) Angiogenetic factor. *THE BONE*, 17, 2, 51-55 (In Japanese)

Saxena, N. K., Taliaferro-Smith, L., Knight, B. B., Merlin, D., Anania, F. A., O’Regan, R. M. & Sharma, D. (2008) Bidirectional crosstalk between leptin and insulin-like growth factor-I signaling promotes invasion and migration of breast cancer cells via transactivation of epidermal growth factor receptor. *Cancer Res*, 68, 23, 9712-9722

Schneider, J. G., Amend, S. R. & Weilbaecher, K. N. (2011) Integrins and bone metastasis: integrating tumor cell and stromal cell interactions. *Bone*, 48, 1, 54-65

Schwaninger, R., Rentsch, C. A., Wetterwald, A., van der Horst, G., van Bezooijen, R. L., van der Pluijm, G., Lowik, C. W., Ackermann, K., Pyerin, W., Hamdy, F. C., Thalmann, G. N. & Cecchini, M. G. (2007) Lack of noggin expression by cancer cells is a determinant of the osteoblast response in bone metastases. *Am J Pathol*, 170, 1, 160-175

Segarini, P. R., Nesbitt, J. E., Li, D., Hays, L. G., Yates, J. R. 3rd & Carmichael, D. F. (2001) The low density lipoprotein receptor-related protein/alpha2-macroglobulin receptor is a receptor for connective tissue growth factor. *J Biol Chem*, 276, 44, 40659-40667

Shakunaga, T., Ozaki, T., Ohara, N., Asaumi, K., Doi, T., Nishida, K., Kawai, A., Nakanishi, T., Takigawa, M. & Inoue, H. (2000) Expression of connective tissue growth factor in cartilaginous tumors. *Cancer*, 89, 7, 1466-1473

Shaw, R. J., Brown, J. S., Woolgar, J. A., Lowe, D., Rogers, S. N. & Vaughan, E. D. (2004) The influence of the pattern of mandibular invasion on recurrence and survival in oral squamous cell carcinoma. *Head Neck*, 26, 10, 861-869

Shi-Wen, X., Leask, A. & Abraham, D. (2008) Regulation and function of connective tissue growth factor/CCN2 in tissue repair, scarring and fibrosis. *Cytokine Growth Factor Rev*, 19, 2, 133-144

Shibahara, T., Nomura, T., Cui, N. H. & Noma, H. (2005) A study of osteoclast-related cytokines in mandibular invasion by squamous cell carcinoma. *Int J Oral Maxillofac Surg*, 34, 7, 789-793
Shimo, T., Nakanishi, T., Kimura, Y., Nishida, T., Ishizeki, K., Matsumura, T. & Takigawa, M. (1998) Inhibition of endogenous expression of connective tissue growth factor by its antisense oligonucleotide and antisense RNA suppresses proliferation and migration of vascular endothelial cells. *J Biochem*, 124, 1, 130-140

Shimo, T., Nakanishi, T., Nishida, T., Asano, M., Kanyama, M., Kuboki, T., Tamatani, T., Tezuka, K., Takemura, M., Matsumura, T. & Takigawa, M. (1999) Connective tissue growth factor induces the proliferation, migration, and tube formation of vascular endothelial cells in vitro, and angiogenesis in vivo. *J Biochem*, 126, 1, 137-145

Shimo, T., Kubota, S., Kondo, S., Nakanishi, T., Sasaki, A., Mesé, H., Matsumura, T. & Takigawa, M. (2001a) Connective tissue growth factor as a major angiogenic agent that is induced by hypoxia in a human breast cancer cell line. *Cancer Lett*, 174, 1, 57-64

Shimo, T., Nakanishi, T., Nishida, T., Asano, M., Sasaki, A., Kanyama, M., Kuboki, T., Matsumura, T. & Takigawa, M. (2001b) Involvement of CTGF, a hypertrophic chondrocyte-specific gene product, in tumor angiogenesis. *Oncology*, 61, 4, 315-322

Shimo, T., Wu, C., Billings, P. C., Piddington, R., Rosenbloom, J., Pacifici, M. & Koyama, E. (2002) Expression, gene regulation, and roles of Fisp12/CTGF in developing tooth germs. *Dev Dyn*, 224, 3, 267-278

Shimo, T., Kanyama, M., Wu, C., Sugito, H., Billings, P. C., Abrams, W. R., Rosenbloom, J., Iwamoto, M., Pacifici, M. & Koyama, E. (2004) Expression and roles of connective tissue growth factor in Meckel’s cartilage development. *Dev Dyn*, 231, 1, 136-147

Shimo, T., Koyama, E., Sugito, H., Wu, C., Shimo, S. & Pacifici, M. (2005) Retinoid signaling regulates CTGF expression in hypertrophic chondrocytes with differential involvement of MAP kinases. *J Bone Miner Res*, 20, 5, 867-877

Shimo, T., Kubota, S., Yoshioka, N., Ibaragi, S., Isowa, S., Eguchi, T., Sasaki, A. & Takigawa, M. (2006) Pathogenic role of connective tissue growth factor (CTGF/CCN2) in osteolytic metastasis of breast cancer. *J Bone Miner Res*, 21, 7, 1045-1059

Shimo, T., Kubota, S., Goda, T., Yoshihama, Y., Kurio, N., Nishida, T., Ng, P. S., Endo, K., Takigawa, M. & Sasaki, A. (2008) Clinical significance and pathogenic function of connective tissue growth factor (CTGF/CCN2) in osteolytic mandibular squamous cell carcinoma. *Anticancer Res*, 28, 4C, 2343-2348

Shimo, T. & Sasaki, A. (2011) Mechanism of cancer-induced bone destruction: An association of connective tissue growth factor (CTGF/CCN2) in the bone metastasis. *Japanese Dental Science Review*, 47, 1, 13-22

Shinto, O., Yashiro, M., Kawajiri, H., Shimizu, K., Shimizu, T., Miwa, A. & Hirakawa, K. (2010) Inhibitory effect of a TGFbeta receptor type-I inhibitor, Ki26894, on invasiveness of scirrhous gastric cancer cells. *Br J Cancer*, 102, 844-51.

Si, W., Kang, Q., Luu, H. H., Park, J. K., Luo, Q., Song, W. X., Jiang, W., Luo, X., Li, X., Yin, H., Montag, A. G., Haydon, R. C. & He, T. C. (2006) CCN1/Cyr61 is regulated by the canonical Wnt signal and plays an important role in Wnt3A-induced osteoblast differentiation of mesenchymal stem cells. *Mol Cell Biol*, 26, 8, 2955-2964
Role of Connective Tissue Growth Factor (CTGF/CCN2) in Oral Squamous Cell Carcinoma-Induced Bone Destruction

Sloan, E. K., Pouliot, N., Stanley, K. L., Chia, J., Moseley, J. M., Hards, D. K. & Anderson, R. L. (2006) Tumor-specific expression of alphavbeta3 integrin promotes spontaneous metastasis of breast cancer to bone. *Breast Cancer Res*, 8, 2, R20

Smerdel-Ramoya, A., Zanotti, S., Stadmeyer, L., Durant, D. & Canalis, E. (2008) Skeletal overexpression of connective tissue growth factor impairs bone formation and causes osteopenia. *Endocrinology*, 149, 9, 4374-4381

Song, J., Chang, L., Chen, Z., Kang, M. & Wang, C. Y. (2010) Characterization of side populations in HNSCC: highly invasive, chemoresistant and abnormal Wnt signaling. *PLoS One*, 5, 7, e11456

Sun, L. (2004) Tumor-suppressive and promoting function of transforming growth factor beta. *Front Biosci*, 9, 1925-1935

Takayama, S., Ishii, S., Ikeda, T., Masamura, S., Doi, M. & Kitajima, M. (2005) The relationship between bone metastasis from human breast cancer and integrin alpha(v)beta3 expression. *Anticancer Res*, 25, 1A, 79-83

Takayama, Y., Mori, T., Nomura, T., Shibahara, T. & Sakamoto, M. (2010) Parathyroid-related protein plays a critical role in bone invasion by oral squamous cell carcinoma. *Int J Oncol*, 36, 6, 1387-1394

Takeshita, S., Kaji, K. & Kudo, A. (2000) Identification and characterization of the new osteoclast progenitor with macrophage phenotypes being able to differentiate into mature osteoclasts. *J Bone Miner Res*, 15, 8, 1477-1488

Takigawa, M., Nakanishi, T., Kubota, S. & Nishida, T. (2003) Role of CTGF/HCS24/ecogenin in skeletal growth control. *J Cell Physiol*, 194, 3, 256-266

Tian, E., Zhan, F., Walker, R., Rasmussen, E., Ma, Y., Barlogie, B. & Shaughnessy, J., Jr. (2003) The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med*, 349, 26, 2483-2494

Van Beek, J. P., Kennedy, L., Rockel, J. S., Bernier, S. M. & Leask, A. (2006) The induction of CCN2 by TGFbeta1 involves Ets-1. *Arthritis Res Ther*, 8, 2, R36

Van der Velde-Zimmermann, D., Verdaasdonk, M. A., Rademakers, L. H., De Weger, R. A., Van den Tweel, J. G. & Joling, P. (1997) Fibronectin distribution in human bone marrow stroma: matrix assembly and tumor cell adhesion via alpha5 beta1 integrin. *Exp Cell Res*, 230, 1, 111-120

Wahab, N. A., Weston, B. S. & Mason, R. M. (2005) Connective tissue growth factor CCN2 interacts with and activates the tyrosine kinase receptor TrkA. *J Am Soc Nephrol*, 16, 2, 340-351

Wang, M. Y., Chen, P. S., Prakash, E., Hsu, H. C., Huang, H. Y., Lin, M. T., Chang, K. J. & Kuo, M. L. (2009) Connective tissue growth factor confers drug resistance in breast cancer through concomitant up-regulation of Bcl-xL and cIAP1. *Cancer Res*, 69, 8, 3482-3491

Wenger, C., Ellenrieder, V., Alber, B., Lacher, U., Menke, A., Hameister, H., Wilda, M., Iwamura, T., Beger, H. G., Adler, G. & Gress, T. M. (1999) Expression and differential regulation of connective tissue growth factor in pancreatic cancer cells. *Oncogene*, 18, 4, 1073-1080

Xie, D., Nakachi, K., Wang, H., Elashoff, R. & Koeffler, H. P. (2001) Elevated levels of connective tissue growth factor, WISP-1, and CYR61 in primary breast cancers associated with more advanced features. *Cancer Res*, 61, 24, 8917-8923

www.intechopen.com
Xu, S. W., Howat, S. L., Renzoni, E. A., Holmes, A., Pearson, J. D., Dashwood, M. R., Bou-Gharios, G., Denton, C. P., du Bois, R. M., Black, C. M., Leask, A. & Abraham, D. J. (2004) Endothelin-1 induces expression of matrix-associated genes in lung fibroblasts through MEK/ERK. *J Biol Chem*, 279, 22, 23098-23103

Yang, D. H., Kim, H. S., Wilson, E. M., Rosenfeld, R. G. & Oh, Y. (1998 Jul;) Identification of glycosylated 38-kDa connective tissue growth factor (IGFBP-related protein 2) and proteolytic fragments in human biological fluids, and up-regulation of IGFBP-rP2 expression by TGF-beta in Hs578T human breast cancer cells. *J Clin Endocrinol Metab*, 83, 7, 2593-2596

Yin, J. J., Mohammad, K. S., Käkönen, S. M., Harris, S., Wu-Wong, J. R., Wessale, J. L., Padley, R. J., Garrett, I. R., Chirgwin, J. M. & Guise, T. A. (2003) A causal role for endothelin-1 in the pathogenesis of osteoblastic bone metastases. *Proc Natl Acad Sci U S A*, 100, 19, 10954-10959

Zambonin Zallone, A., Teti, A., Gaboli, M. & Marchisio, P. C. (1989) Beta 3 subunit of vitronectin receptor is present in osteoclast adhesion structures and not in other monocyte-macrophage derived cells. *Connect Tissue Res*, 20, 1-4, 143-149
This book points to some new areas for investigation on squamous cell carcinoma (SCC). Firstly, the features and management of some specific SCC is discussed to give the readers the general principles in dealing with these uncommon and sophisticated conditions. Some new concepts in adjuvant therapy including neoadjuvant therapy and gold nanoparticle-based photo dynamic therapy are introduced. Secondly, a detailed discussion of molecular aspects of tumor invasion and progression in SCC is provided with the emphasis on the roles of some important factors. The role of tumor microenvironment in head and neck SCC is specifically discussed. Thirdly, the roles of cancer stem cells (CSC) in cancer therapy of SCC are described. Molecular mechanisms involving therapeutic resistance and new therapeutic strategies targeting CSC are discussed in detail. Finally, other aspects concerning SCC are included, which involve the assessment, genetic manipulation and its possible clinical implications for the treatment of SCC.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Tsuyoshi Shimo and Akira Sasaki (2012). Role of Connective Tissue Growth Factor (CTGF/CCN2) in Oral Squamous Cell Carcinoma-Induced Bone Destruction, Squamous Cell Carcinoma, Prof. Xiaoming Li (Ed.), ISBN: 978-953-51-0024-9, InTech, Available from: http://www.intechopen.com/books/squamous-cell-carcinoma/the-role-of-connective-tissue-growth-factor-ctgf-ccn2-in-oral-squamous-cell-carcinoma-induced-bone-d
