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TOPIC HIGHLIGHT

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Inhibition of rheumatoid arthritis by blocking connective tissue growth factor

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Abstract

The pathogenesis of rheumatoid arthritis (RA) remains to be completely elucidated so far; however, it is known that proinflammatory cytokines play a pivotal role in the induction of RA. Tumor necrosis factor (TNF-α), in particular, is considered to play a central role in bone destruction by mediating the abnormal activation of osteoclasts or the production of proteolytic enzymes through direct or indirect mechanisms. The use of TNF-α blocking agents has a significant impact on RA therapy. Anti-TNF-α blocking agents such as infliximab are very effective for treatment of RA, especially for the prevention of articular destruction. We have previously shown that several proteins exhibited extensive changes in their expression after amelioration of RA with infliximab treatment. Among the proteins, connective tissue growth factor (CTGF) has a significant role for the development of RA. Herein, we review the function of CTGF in the pathogenesis of RA and discuss the possibility of a novel treatment for RA. We propose that CTGF is a potentially novel effector molecule in the pathogenesis of RA. Blocking the CTGF pathways by biological agents may have great beneficial effect in patients with RA.

Key words: Connective tissue growth facto; Rheumatoid arthritis; Osteoclasts; Condrocytes; Tumor necrosis factor-α

Core tip: Connective tissue growth factor (CTGF) plays an important role in the pathogenesis of rheumatoid arthritis (RA). We propose that CTGF is a potentially novel effector molecule in the pathogenesis of RA. Blocking the CTGF pathways by biological agents may have great beneficial effect in patients with RA.

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INTRODUCTION

Rheumatoid arthritis (RA) causes chronic inflammation and consequently destruction of the articular tissue. Although the pathogenesis of RA are not fully understood, proinflammatory cytokines such as tumor necrosis factor (TNF)-α have been proposed as important factors in the pathogenesis of RA[1-4]. TNF-α is multiple functional cytokine. In addition to inflammatory process, TNF-α concerns various physiological phenomena in RA[5,6]. Moreover, accumulating reports suggest that TNF-α

Table 1 Proteins with greater changes after infliximab treatment[1]

	Molecular weight	pI	Accession#
Peptidylprolyl isomerase B precursor	23728	9.42	NP_000933
Caldesmon 1 isoform 1	93175	5.62	NP_149129
Family with sequence similarity 62 (C2 domain containing), member A	122780	5.57	NP_056107
Amylase, alpha 2A; pancreatic precursor	57670	6.60	NP_000690
Filamin 1 (actin-binding protein-280)	280586	5.73	NP_001447
Vasodilator-stimulated phosphoprotein isoform 1	39805	9.05	NP_003361
Cycteine and glycine-rich protein 1	20554	8.90	NP_004069
Myoglobin	17173	7.14	NP_005359
Transgelin 2	22377	8.41	NP_003555
Microtubule-associated protein, RP/EB family, member 1	29980	5.02	NP_036457
NCK adaptor protein isoform A	42889	6.49	NP_003572
Tropomudulin 3 (ubiquitous)	39570	5.08	NP_055362
Connective tissue growth factor	38043	8.36	NP_001892
Latent transforming growth factor beta binding protein 1 isoform	186716	5.63	NP_996826
Regenerating islet-derived 1 alpha precursor	18719	5.65	NP_002900
Peptidylprolyl isomerase A isofoem 1	18001	7.68	NP_066953
Coronin, actin binding protein, 1C	53215	6.65	NP_055140
Triggering receptor expressed on myeloid cells-like 1	32658	5.70	NP_835468
Heparin sulfate proteoglycan 2	468528	6.06	NP_005520
Peptidoglycan recognition protein 1	21717	8.92	NP_005082
Superoxidase dismutase 1, soluble	15926	5.70	NP_000445

promotes bone destruction in RA, as excess TNF-α cause the abnormal osteoclaststic activation by direct or indirect interaction[2,3]. Our previous study used a novel approach of proteomic research and showed a significant profile change of serum protein biomarkers (approximately 20 proteins) in patients with RA treated using infliximab[1]. Among the proteins listed in our previous study, we found that connective tissue growth factor (CTGF) played an important role for the amelioration of RA[7-9] patients in infliximab treatment (Table 1). Based on this finding, we undertook subsequent studies to analyze the contribution of CTGF in the pathogenesis of RA and found that it plays an important role[8-10]. Herein, we review the function of CTGF in the pathogenesis of RA based on these findings. In RA, aberrant CTGF regulation may induce aberrant osteoclastogenesis and cause disturbance in cartilage homeostasis, subsequently resulting in articular tissue destruction. Blocking the CTGF pathways may be a novel effective strategy in the treatment of RA.

CONNECTIVE TISSUE GROWTH FACTOR

CTGF was originally identified in human umbilical endothelial cell supernatants that exhibit platelet-derivedgrowth factor (PDGF)-like chemotactic and mitogenic activities toward mesenchymal cells; the cDNA was isolated from a human vein endothelial cells (HUVECs) cDNA expression library using anti-PDGF and it encoded a 349-amino acid protein[11]. CTGF belongs to the CCN protein family and is believed to be a downstream molecule of transforming growth factor (TGF)-β pathway[12]. Although several candidate specific CTGF receptors have been currently proposed, they have not yet been completely identified to date. CTGF is associated with several biological functions such as fibrosis, tumorigenesis, angiogenesis, and endochondral ossification[13]. CTGF in articular tissue, consisting different types of cells, is produced by chondrocytes and maintains cartilage tissue homeostasis *via* the autocrine process. Furthermore, incomplete knock-down of the CTGF gene dramatically inhibits osteoclast-like cell formation in mice, even though the complete knock-down mice exhibit embryonic lethality[14].

CONTRIBUTION OF CTGF TO THE PROGRESSION OF RA

In vivo transfection with an adenovirus expression vector that encodes CTGF into mouse knee joints has been shown to cause cartilage damage due to an increase in mRNA coding for proteolytic enzymes such as matrix metalloproteinase (MMP)-3[15]. Manns *et al*[16] reported the up-regulation of CTGF in an experimental animal model of RA; treatment with a thrombospondin-1-derived peptide ameliorate the development of arthritis concomitant with the down-regulation of CTGF. These reports have indicated that CTGF has a significant role in the pathogenesis of RA. In addition, we observed the following interesting findings in our previous studies: (1) CTGF was overproduced by synovial fibroblasts in patients with RA (Figure 1); (2) the production of CTGF was regulated by TNF-α. CTGF production was up-regulated in synovial fibroblasts and down-regulated in chondrocytes (Figure 2); and (3) CTGF in combination with MCSF/RANKL promoted osteoclastogenesis (Figure 3)[8]. In the results of our study, we observed that TNF-α induced CTGF production by synovial cells. In contrast, TNF-α inhibited CTGF production by chondrocytes. TNF receptors have shown to transduce and amplify receptor activation resulting different cellular fates such as NF-κB activation or apoptosis. Although precise intracellular mechanisms

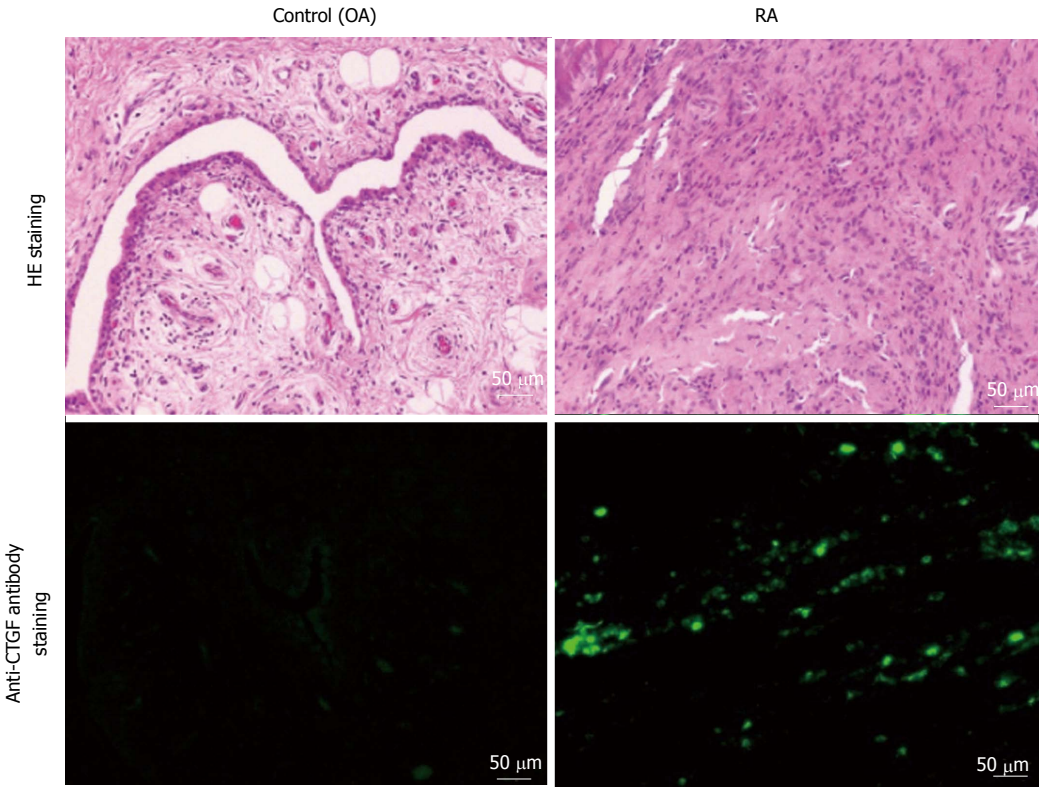

Figure 1 Connective tissue growth factor expression was increased at synovial tissue in rheumatoid arthritis. Representative results of hematoxylin and eosin (HE) staining, immunofluorescence anti-connective tissue growth factor (CTGF) antibody staining, and anti-F4/80 antibody staining are shown using surgical samples from patients with rheumatoid arthritis (RA) and osteoarthritis (OA). The observed CTGF expression was stronger in the samples of patients with RA than in the samples of patients with OA.

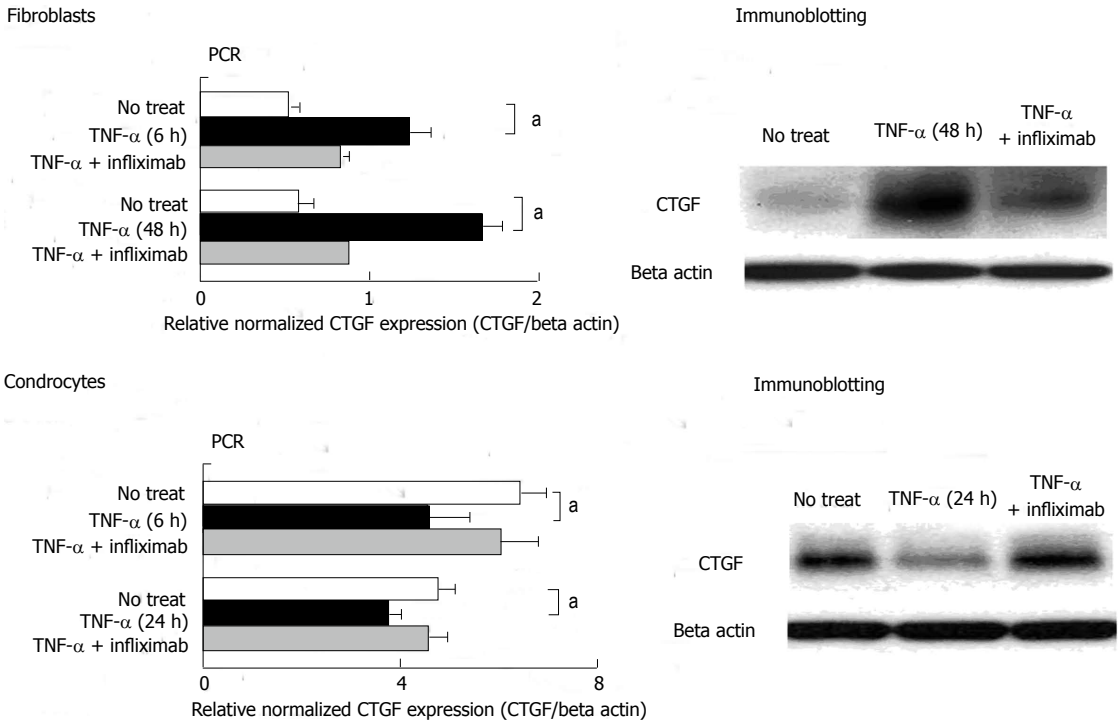

Figure 2 Tumor necrosis factor-α positively regulated connective tissue growth factor production in synovial fibroblasts and negatively regulated connective tissue growth factor production in chondrocytes. Connective tissue growth factor (CTGF) production from the human synovial fibroblasts cell line (MH7A) and the human chondrocytes cell line (OUMS-27) stimulated with/without tumor necrosis factor (TNF)-α were evaluated by immunoblotting and quantitative real time polymerase chain reaction (PCR). TNF-α promoted CTGF production by synovial fibroblasts and inhibited the production by chondrocytes. Statistical analysis (paired *t* test) was performed, and $^{a}P < 0.05$ were considered to be statistically significant. $^{a}P < 0.05$, TNF-α *vs* no treat.

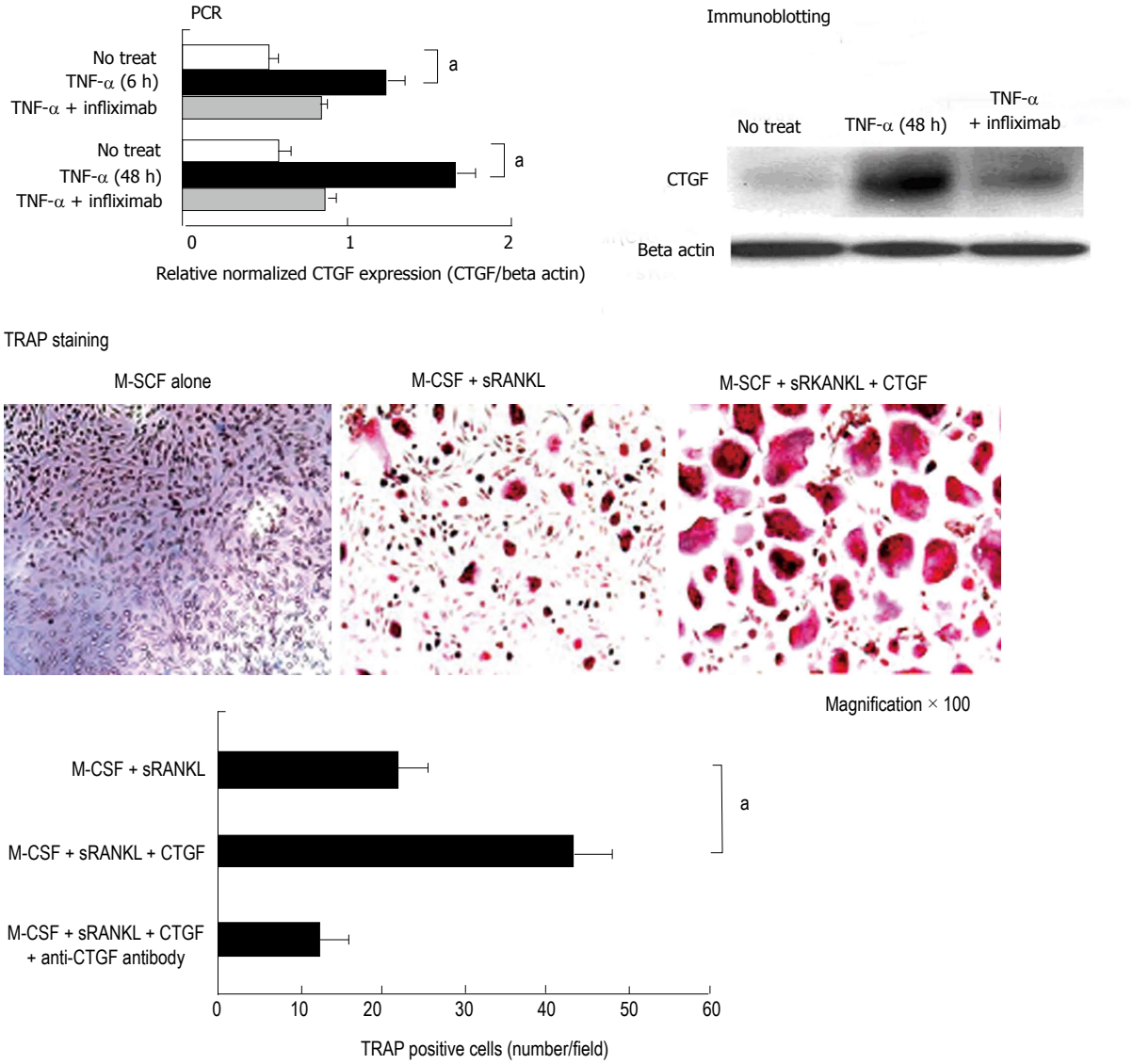

Figure 3 Connective tissue growth factor enhanced macrophage-colony stimulating factor/ receptor activator of nuclear factor kappa-B ligand mediated osteoclastgenesis. Figure 3 Shows images of tartrate-resistant acidic phosphatase (TRAP) staining and the number of TRAP positive cells. For the evaluation of osteoclastogenesis, $CD14^+$ were purified from peripheral blood mononuclear cells of healthy volunteers to obtain osteoclastic progenitor cells. Osteoclasts were induced with macrophage-colony stimulating factor (M-CSF) and soluble receptor activator of nuclear factor kappa-B ligand (sRANKL) and the osteoclastgenesis was evaluated by TRAP staining. The TRAP positive cells were defined as osteoclasts. Connective tissue growth factor (CTGF) alone could not help in the differentiation of osteoclasts (data not shown). M-CSF/RANKL-mediated osteoclastogenesis was enhanced, which was detected by the production of CTGF by larger and higher number of osteoclasts; this enhancing effect was abolished by anti-CTGF antibody. The bars in Figure 2 indicate the standard deviation. Statistical analysis (paired *t* test) was performed, and *P* values < 0.05 were considered to be statistically significant. $^aP < 0.05$, M-CSF + sRANKL + CTGF *vs* M-CSF + sRANKL.

has not elucidated, previous studies have indicated that TNF-α increased or inhibited CTGF production depend on cell types. For example, TNF-α positively regulated CTGF production in mesangial cells[17]. On the other hand, TNF-α negatively regulated CTGF production in human lung endothelial cells[18].

CTGF has been suggested to contribute to the homeostasis of cartilaginous tissue by autocrine process[14]. CTGF also may positively regulate proliferation of osteoblasts[14], Therefore, CTGF may function as positive regulator functions for proliferation of chondrocytes and osteoblasts, consequently remaining the physiological articular tissue homeostasis. The disturbance of homeostasis due to impairment of CTGF production from chondrocytes possibly result in cartilage tissue damage in RA. However, our data indicated that TNF-α was able to stimulate CTGF production in synovial fibroblasts. The excessive CTGF produced by synovial fibroblasts logically may function as protective factor for cartilage destruction in RA, because CTGF plays an important role for chondrogenesis. On the other hand, TNF-α has shown to induce catalytic enzymes production such as MMPs which cause cartilage destruction in synovial fibroblasts. Moreover, our data also indicated that TNF-α oppositely inhibited CTGF production in condrocytes. In RA, TNF-α possibly functions as positive regulator for cartilage destruction through catalytic enzymes production or the inhibition of CTGF production in condrocytes more efficiently rather than functions as negative regulator for cartilage destruction through increased CTGF produc-

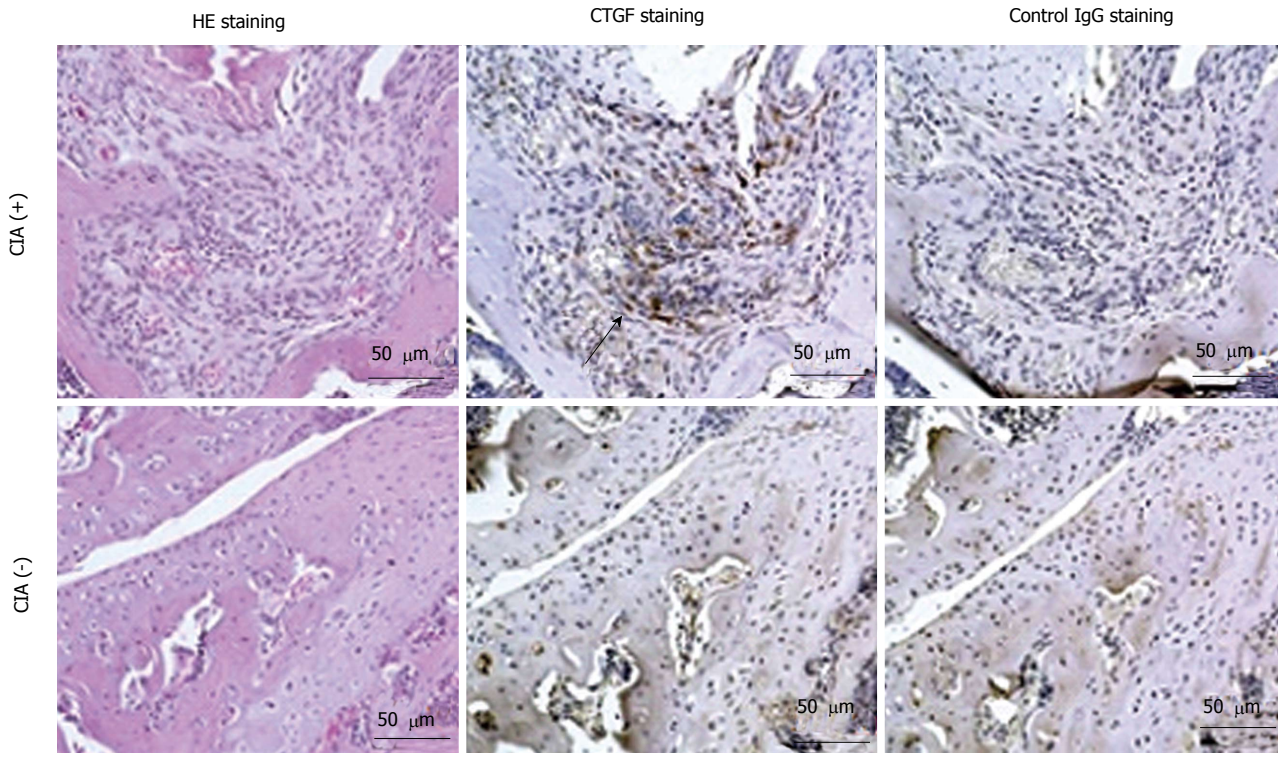

Figure 4 Increased *in vivo* expression of connective tissue growth factor at the articular tissue in collagen-induced arthritis mice. The collagen-induced arthritis (CIA) mice were sacrificed at 8 wk after immunization for immunohistochemical analysis. The immunohistochemical staining showed massive connective tissue growth factor (CTGF) expression in the articular tissue samples from CIA mice (indicated by arrow) using anti-CTGF antibody or control goat immunoglobulin G antibody. Serial sections of the articular tissue samples were also counterstained with hematoxylin/eosin (HE) for detection of the arthritis.

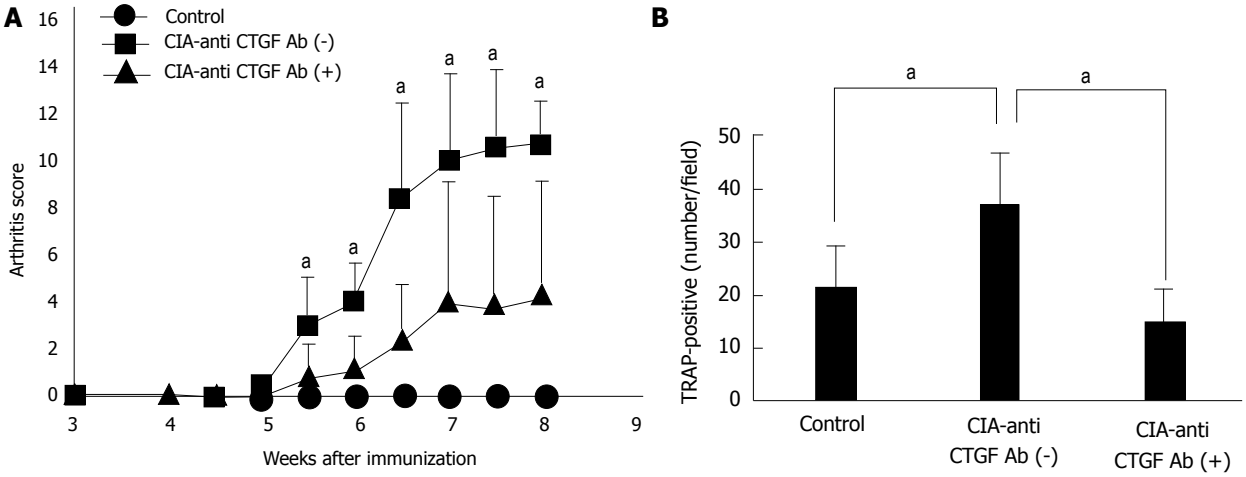

Figure 5 Blocking connective tissue growth factor prevented the development of arthritis in collagen-induced arthritis mice. Mice with collagen-induced arthritis (CIA) were randomly selected and were intraperitoneally administered every week with anti-connective tissue growth factor (CTGF) monoclonal antibodies (mAbs) (white triangle; CIA-anti-CTGF Ab+) or control purified immunoglobulin (white square; CIA-anti-CTGF Ab−) from 1 wk before immunization to 6 weeks after immunization. Each group comprised 12 mice. The mice were monitored for arthritis every week and scored in a blinded manner (A). Blocking CTGF could efficiently prevent the development of CIA in mice. Bars in Figure 3 indicate the standard deviation. Statistical analysis (anti-CTGF Ab$^+$ *vs* anti-CTGF Ab−) was performed, and *P* values < 0.05 were considered to be statistically significant. ^{a}*P* < 0.05, CIA anti-CTGF Ab *vs* Control. For evaluation of osteoclastogenesis, CD14$^+$ osteoclastic progenitor cells were purified from splenocytes at 8 wk after immunization and osteoclasts were then induced with macrophage-colony stimulating factor (M-CSF) and soluble receptor activator of nuclear factor kappa-B ligand (sRANKL). Osteoclastogenesis was suppressed in mice with CIA treated using anti-CTGF mAb (B) compared to the non-treated mice. The bars in Figure 3 indicate the standard deviation. Statistical analysis was performed, and *P* values < 0.05 were considered to be statistically significant. ^{a}*P* < 0.05, CIA anti-CTGF Ab (-) *vs* Control; CIA anti-CTGF Ab (-) *vs* CIA anti-CTGF Ab (+).

tion in synovial fibroblasts. Taken together, excessive CTGF production by synovial fibroblasts regulated by TNF-α promotes aberrant activation of osteoclasts and disturbs the homeostasis of cartilage tissue, ultimately resulting in articular distraction.

Next, we performed an *in vivo* study to clarify the

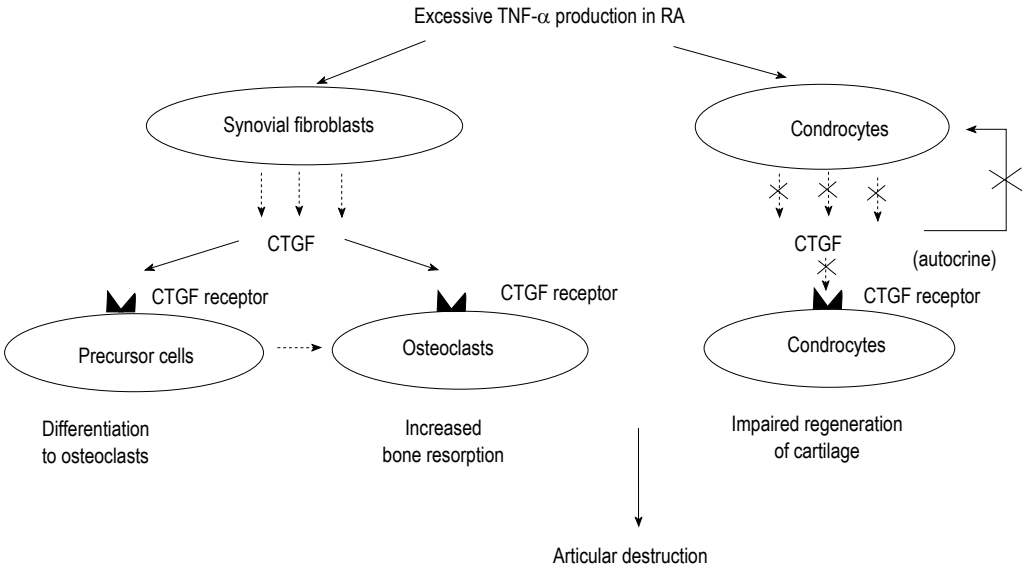

Figure 6 Hypothesis of the role of connective tissue growth factor in the pathogenesis of rheumatoid arthritis. Hypothesis of the possible role of connective tissue growth factor (CTGF) in the pathogenesis of rheumatoid arthritis (RA). TNF: Tumor necrosis factor.

pathological roles of CTGF in the arthritis development using a murine collagen-induced arthritis (CIA) model. A DBA/1J mice were immunized with a combination of type Ⅱ collagen and complete Freund adjuvant (CFA) for induction of CIA. We confirmed *in vivo* CTGF expression was increased at the articular tissue in CIA mice as well as human patients with RA (Figure 4). Moreover, we evaluated the efficacy of the neutralizing anti-CTGF monoclonal antibody (mAb) in the prevention of CIA development in mice. We found that the neutralizing anti-CTGF mAb significantly ameliorated CIA in the treated mice (Figure 5A). In addition, aberrant osteoclastogenesis observed in the mice with CIA was reduced by anti-CTGF mAb treatment (Figure 5B). Our consecutive studies showed that blocking the production of CTGF prevented the progression of RA. Therefore, CTGF may be a new therapeutic target for the treatment of RA.

CONCLUSION

We confirmed that CTGF is a novel effecter molecule in the pathogenesis of RA. A schematic hypothesis of its role is presented in Figure 6. CTGF is a multiple functional cytokines and possess a several biological functions depend on the target cells. Although many candidate molecules on the cell surface have been suggested as specific CTGF receptors such as integrins, they have not been completely identified to date. Biological functions of CTGF may differ depend on its receptor as well as cell types. Although the mechanism of action and the importance of CTGF in contribution to the RA development are unclear, we showed that blocking the CTGF pathway could ameliorate CIA especially through the reduction of aberrant osteoclastogenesis. These data imply the possible mechanism underlying the efficacy of anti-CTGF antibody in the treatment with RA. Our study indicated that CTGF is important factor in the development of RA. These results may shed light on the new therapeutic strategies for RA. Further precise studies that will provide clues to assist in the development of new treatment for RA as well as a deeper understanding of its etiology are required.

REFERENCES

1 **Feldmann M**, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996; **14**: 397-440 [PMID: 8717520 DOI: 10.1146/annurev.immunol.14.1.397]
2 **Fassbender HG**, Seibel M, Hebert T. Pathways of destruction in metacarpal and metatarsal joints of patients with rheumatoid arthritis. *Scand J Rheumatol* 1992; **21**: 10-16 [PMID: 1570480 DOI: 10.3109/03009749209095055]
3 **Raisz LG**. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. *J Clin Invest* 2005; **115**: 3318-3325 [PMID: 16322775 DOI: 10.1172/JCI27071]
4 **Teitelbaum SL**. Osteoclasts: what do they do and how do they do it? *Am J Pathol* 2007; **170**: 427-435 [PMID: 17255310 DOI: 10.2353/ajpath.2007.060834]
5 **Maini RN**, Elliott MJ, Brennan FM, Williams RO, Chu CQ, Paleolog E, Charles PJ, Taylor PC, Feldmann M. Monoclonal anti-TNF alpha antibody as a probe of pathogenesis and therapy of rheumatoid disease. *Immunol Rev* 1995; **144**: 195-223 [PMID: 7590814 DOI: 10.1111/j.1600-065X.1995.tb00070.x]
6 **Redlich K**, Hayer S, Ricci R, David JP, Tohidast-Akrad M, Kollias G, Steiner G, Smolen JS, Wagner EF, Schett G. Osteoclasts are essential for TNF-alpha-mediated joint destruction. *J Clin Invest* 2002; **110**: 1419-1427 [PMID: 12438440 DOI: 10.1172/JCI0215582]
7 **Sekigawa I**, Yanagida M, Iwabuchi K, Kaneda K, Kaneko H, Takasaki Y, Jung G, Sone S, Tanaka Y, Ogawa H, Takamori K. Protein biomarker analysis by mass spectrometry in patients with rheumatoid arthritis receiving anti-tumor necrosis factor-alpha antibody therapy. *Clin Exp Rheumatol* 2008; **26**: 261-267 [PMID: 18565247]
8 **Nozawa K**, Fujishiro M, Kawasaki M, Kaneko H, Iwabuchi K, Yanagida M, Suzuki F, Miyazawa K, Takasaki Y, Ogawa H, Takamori K, Sekigawa I. Connective tissue growth factor promotes articular damage by increased osteoclastogenesis

in patients with rheumatoid arthritis. *Arthritis Res Ther* 2009; **11**: R174 [PMID: 19922639 DOI: 10.1186/ar2863]

9 **Nozawa K**, Fujishiro M, Kawasaki M, Yamaguchi A, Ikeda K, Morimoto S, Iwabuchi K, Yanagida M, Ichinose S, Morioka M, Ogawa H, Takamori K, Takasaki Y, Sekigawa I. Inhibition of connective tissue growth factor ameliorates disease in a murine model of rheumatoid arthritis. *Arthritis Rheum* 2013; **65**: 1477-1486 [PMID: 23436223]

10 **Fujishiro M**, Yamaguchi A, Kawasaki M, Nozawa K, Takasaki Y, Takamori K, Ogawa H, Endo K, Ng PS, Takehara K, Sekigawa I. The detection of plasma levels of connective tissue growth factor in rheumatoid arthritis patients. *Clin Exp Rheumatol* 2012; **30**: 145-146 [PMID: 22244584]

11 **Bradham DM**, Igarashi A, Potter RL, Grotendorst GR. 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* 1991; **114**: 1285-1294 [PMID: 1654338 DOI: 10.1083/jcb.114.6.1285]

12 **Grotendorst GR.** Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts. *Cytokine Growth Factor Rev* 1997; **8**: 171-179 [PMID: 94622483 DOI: 10.1016/S1359-6101(97)00010-5]

13 **Perbal B**. CCN proteins: multifunctional signalling regulators. *Lancet* 2004; **363**: 62-64 [PMID: 14723997 DOI: 10.1016/S0140-6736(03)15172-0]

14 **Takigawa M**, Nakanishi T, Kubota S, Nishida T. Role of CTGF/HCS24/ecogenin in skeletal growth control. *J Cell Physiol* 2003; **194**: 256-266 [PMID: 12548546 DOI: 10.1002/jcp.10206]

15 **Blaney Davidson EN**, Vitters EL, Mooren FM, Oliver N, Berg WB, van der Kraan PM. Connective tissue growth factor/CCN2 overexpression in mouse synovial lining results in transient fibrosis and cartilage damage. *Arthritis Rheum* 2006; **54**: 1653-1661 [PMID: 16646035 DOI: 10.1002/art.21795]

16 **Manns JM**, Uknis AB, Rico MC, Agelan A, Castaneda J, Arango I, Barbe MF, Safadi FF, Popoff SN, DeLa Cadena RA. A peptide from thrombospondin 1 modulates experimental erosive arthritis by regulating connective tissue growth factor. *Arthritis Rheum* 2006; **54**: 2415-2422 [PMID: 16869004 DOI: 10.1002/art.22021]

17 **Cooker LA**, Peterson D, Rambow J, Riser ML, Riser RE, Najmabadi F, Brigstock D, Riser BL. TNF-alpha, but not IFN-gamma, regulates CCN2 (CTGF), collagen type I, and proliferation in mesangial cells: possible roles in the progression of renal fibrosis. *Am J Physiol Renal Physiol* 2007; **293**: F157-F165 [PMID: 17376761 DOI: 10.1152/ajprenal.00508.2006]

18 **Laug R**, Fehrholz M, Schütze N, Kramer BW, Krump-Konvalinkova V, Speer CP, Kunzmann S. IFN-γ and TNF-α synergize to inhibit CTGF expression in human lung endothelial cells. *PLoS One* 2012; **7**: e45430 [PMID: 23029004 DOI: 10.1371/journal.pone.0045430]

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