Inhibition of rheumatoid arthritis by blocking connective tissue growth factor

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Author contributions: All authors contributed to this paper.

Supported by Grant-in-Aid for Scientific Research (C), The Ministry of Education, Culture, Sports, Science and Technology and The Institute for Environment and Gender-specific Medicine, Juntendo University Graduate School of Medicine

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Received: December 26, 2013 Revised: April 14, 2014
Accepted: July 17, 2014
Published online: November 18, 2014

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.

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Key words: Connective tissue growth factor; Rheumatoid arthritis; Osteoclasts; Chondrocytes; 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.

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. TNF-α is multiple functional cytokine. In addition to inflammatory process, TNF-α concerns various physiological phenomena in RA. Moreover, accumulating reports suggest that TNF-α...
promotes bone destruction in RA, as excess TNF-α cause the abnormal osteoclastic activation by direct or indirect interaction\textsuperscript{[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\textsuperscript{[8-10]}. 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\textsuperscript{[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\textsuperscript{[11]} in RA patients treated with infliximab. In our study, we observed that TNF-α promoted osteoclastogenesis (Figure 3); (2) the production of CTGF was regulated by TNF-α promotion of osteoclastogenesis 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). 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

### Table 1 Proteins with greater changes after infliximab treatment\textsuperscript{[11]}

| Accession# | Molecular weight | pI | Peptide/protein isoform |
|------------|----------------|----|------------------------|
| NP_001892  | 23728          | 9.42| NP_000933 |
| NP_005520  | 57670          | 6.60| NP_000690 |
| NP_001447  | 186716         | 5.63| NP_001892 |
| NP_003361  | 17173          | 7.14| NP_005359 |
| NP_002900  | 22377          | 8.41| NP_003555 |
| NP_000933  | 20554          | 8.90| NP_004069 |
| NP_005362  | 29980          | 5.02| NP_036457 |
| NP_0035362 | 32658          | 7.68| NP_066953 |
| NP_055140  | 35215          | 6.65| NP_055140 |
| NP_053520  | 36843          | 8.36| NP_001892 |
| NP_039570  | 37819          | 5.65| NP_002800 |
| NP_149129  | 18001          | 7.68| NP_066953 |
| NP_053520  | 32658          | 7.68| NP_066953 |
| NP_004069  | 35215          | 6.65| NP_055140 |
| NP_004069  | 37819          | 5.65| NP_002800 |
| NP_0035362 | 32658          | 7.68| NP_066953 |
| NP_0035362 | 35215          | 6.65| NP_055140 |
| NP_005359  | 18001          | 7.68| NP_066953 |
| NP_055140  | 32658          | 7.68| NP_066953 |
| NP_036457  | 29980          | 5.02| NP_036457 |
| NP_036457  | 29980          | 5.02| NP_036457 |
| NP_053520  | 32658          | 7.68| NP_066953 |
| NP_053520  | 32658          | 7.68| NP_066953 |
| NP_053520  | 32658          | 7.68| NP_066953 |

### CONNECTIVE TISSUE GROWTH FACTOR

CTGF was originally identified in human umbilical endothelial cell supernatants that exhibit platelet-derived growth 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 beta actin binding protein, 1C. 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\textsuperscript{[13-14]}. 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\textsuperscript{[15]}. 

### 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)-13\textsuperscript{[16]}. Manne et al\textsuperscript{[17]} 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; (3) CTGF production was up-regulated in synovial fibroblasts and down-regulated in chondrocytes (Figure 2); and (4) CTGF in combination with MCSF/RANKL promoted osteoclastogenesis (Figure 3). 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 *P < 0.05 were considered to be statistically significant. *P < 0.05, TNF-α vs no treat.
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 increased CTGF production or the inhibition of CTGF production in condrocytes more efficiently rather than functions as negative regulator for cartilage destruction through increased CTGF production.

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\(^\text{[17]}\). On the other hand, TNF-α negatively regulated CTGF production in human lung endothelial cells\(^\text{[18]}\).

CTGF has been suggested to contribute to the homeostasis of cartilaginous tissue by autocrine process\(^\text{[14]}\). CTGF also may positively regulate proliferation of osteoblasts\(^\text{[14]}\). 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 production.
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
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 effector 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.

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P- Reviewer: Andonopoulos AP, Saviola G S- Editor: Ji FF L- Editor: A E- Editor: Wu HL
