Major Histocompatibility Complex Class II Transactivator Inhibits Cysteine-rich 61 Expression in Osteoblastic Cells and Its Implication in the Pathogenesis of Periapical Lesions

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Abstract

Introduction: Osteoblastic expression of cysteine-rich 61 (Cyr61) correlates with the severity of periapical lesion–associated bone loss, but the regulatory mechanism of Cyr61 expression was not known. **Methods:** In the study we examined the effect of major histocompatibility complex class II transactivator (CIITA) on tumor necrosis factor (TNF)-α–induced Cyr61 synthesis in U2OS human osteoblastic cells by Western blot analysis. In a rat model of bacteria-induced apical periodontitis, we assessed the relation between osteoblastic expressions of CIITA/Cyr61 and disease progression by radiographic and immunohistochemistry studies. **Results:** We found that forced expression of CIITA suppressed Cyr61 synthesis in U2OS cells. In rat periapical lesions, osteoblastic CIITA decreased as the disease progressed, and expression of CIITA is negatively related to Cyr61 synthesis in osteoblasts. **Conclusions:** Our data showed that CIITA is a repressor of Cyr61 synthesis in osteoblasts, and it might play a protective role in the pathogenesis of bone resorption in apical periodontitis, possibly through down-regulating the expression of Cyr61 in osteoblasts. *(J Endod 2010;36:1021–1025)*

Key Words

Apical periodontitis, bone resorption, CIITA, Cyr61, osteoblasts

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Periapical lesions are primarily a consequence of the presence of bacteria in the root canals. Continuous flow of bacteria and their products through the apical foramen induces both specific and nonspecific immune responses within the periapical area and ultimately results in bone destruction (1). The pathologic process is characterized by the activation of immunocompetent cells that produce a wide variety of inflammatory mediators (2). Tumor necrosis factor (TNF)-α is one of the major proinflammatory cytokines present in periapical lesions (3, 4), and it is able to induce other inflammatory mediators and proteases that orchestrate inflammatory responses (5, 6). In previous studies we have demonstrated the role of TNF-α in the development of apical periodontitis and concomitant bone resorption (7–9).

Named after 3 prototypical members, cysteine-rich protein 61 (Cyr61, also known as CCN1), connective tissue growth factor (CTGF, also known as CCN2), and nephroblastoma overexpressed protein (Nov, also known as CCN3), the CCN family comprises 6 members, CCN1 to CCN6 (10, 11). Once synthesized, CCN proteins are secreted, associated with cell surface or extracellular matrix through binding to integrins or heparan sulfate proteoglycans, and serve as matricellular signaling molecules (10, 12). Functionally, Cyr61 has been shown to regulate angiogenesis, cell proliferation, adhesion, migration, and differentiation (10, 11). The proangiogenic and chemotactic activities of Cyr61 suggest that it might play a role in the mediation of inflammation (13, 14). In fact, many studies have implicated Cyr61 in the pathogenesis of inflammatory diseases (12, 15–17). Recently, we showed that Cyr61 expression is up-regulated by TNF-α in human osteoblastic cells (18). Furthermore, in a rat model of bacteria-induced apical periodontitis, we found that osteoblastic expression of Cyr61 correlates with the severity of inflammation-associated bone loss (18, 19). However, the mechanism involved in the regulation of Cyr61 expression in osteoblasts was not known.

The major histocompatibility complex (MHC) class II transactivator (CIITA) is the major regulator of expression of MHC class II genes in immune cells. CIITA functions as a non–DNA-binding co-activator that coordinates multiple events that are required for the activation of transcription (20). In recent years, reports indicated that CIITA can influence the expression of a variety of genes that are involved in numerous functions within and outside the immune system (21). DNA microarray analysis identified more than 40 genes that were up-regulated by CIITA in human B cells and in interferon-γ–stimulated cells (22). In contrast, other genes were reported to be repressed by CIITA in specific cell types (22–26). However, the possible role of CIITA in the regulation of gene expression in osteoblasts has not been studied.

In the present study we examined the effect of CIITA on Cyr61 production in human osteoblastic cells and assessed the relation between osteoblastic expression of CIITA and disease activity in a rat model of apical periodontitis. We found that CIITA is a suppressor of Cyr61 synthesis in osteoblasts, and osteoblastic expression of CIITA is negatively related to the progression of periapical lesions.

Materials and Methods

Cell Culture and Treatments

U2OS, a human osteosarcoma cell line with a characteristic osteoblastic phenotype, was maintained in Dulbecco modified Eagle medium (DMEM) supplemented

with 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere of air and 5% CO_2 at 37 °C. To evaluate the effect of TNF-α on the synthesis of Cyr61, U2OS cells were plated at a density of 5×10^5 cells/mL on 10-cm culture dishes and treated with 20 ng/mL of TNF-α for 0, 8, 12, 24, 48, and 72 hours. The action of CIITA on TNF-α–induced Cyr61 expression *in vitro* was evaluated by transfecting U2OS cells with 2 or 5 μg of the CIITA expressing plasmid Myc-CIITA/pcDNA3, a gift from Dr Ting (University of North Carolina at Chapel Hill, Chapel Hill, NC) (23). Briefly, before transfection, cells were plated in 6-well culture plates at a density of 6×10^5 cells per well and incubated overnight. Transfections were performed in serum-free DMEM by using Arrest-In transfection reagent (Open Biosystems Co, Huntsville, AL) according to the manufacturer's protocol. Six hours after transfection, cells were transferred to and incubated in serum-containing DMEM for 48 hours. The transfected cells were treated with 20 ng/mL of TNF-α for 24 hours before further assessment. Independent experiments were performed 3 times.

Western Blot Analysis

Western blot analysis was performed as previously described (18). In brief, cells were lysed in lysis buffer, fractionated by 12.5% sodium dodecylsulfate–polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes by electroblotting. After treatment with antibodies against human/rat Cyr61 (Santa Cruz Biotechnology, Santa Cruz, CA), proteins were visualized by horseradish peroxidase (HRP)–conjugated immunoglobulin and an enhanced chemiluminescence detection system (Amersham, Little Chalfont, Buckinghamshire, UK). The membranes were reprobed with β-actin antibody (Abcam Inc, Cambridge, MA). The results of Western blots were digitized for densitometric analysis by normalizing the data to the β-actin levels.

Animal Model of Periapical Lesions

Periapical lesions were induced in 20 seven- to eight-week-old Sprague-Dawley rats as described previously (18). The experimental protocol was approved by the Center of Laboratory Animal, College of Medicine, National Taiwan University, and the animals were maintained following the Guide to Management and Use of Experimental Animals, National Science Council, Taiwan. On day 0, the animals were anesthetized by intraperitoneal injection of 3 mg of sodium pentobarbital per 100 g body weight. Pulp exposure was performed at the distal fossa of right mandibular first molars by using #1/4 round bur to the depth of bur diameter. The exposed pulps were left open to the oral environment to induce the formation of periapical lesions. The animals were randomly assigned into either small or large lesion groups (n = 10 per group), in which they were killed 10 and 20 days, respectively, after lesion induction.

Radiography and Image Analysis

The jaws were dissected and processed for imaging as described previously (18). Briefly, radiographs were taken and analyzed by Digora image analysis system (Soredex, Helsinki, Finland). The areas of periapical lesions at the distal root apices of the right mandibular first molars were quantified in pixels by using software specially designed by the College of Electrical Engineering, National Taiwan University. Data were transformed to square millimeters by using 1 mm^2 = 256 pixels, as determined by assaying a standard of known area.

Immunohistochemistry

After imaging, the mandibles were fixed in 4% paraformaldehyde at 4°C for 48 hours, decalcified with 14% ethylenediaminetetraacetic acid (pH 7.1) for 2 weeks, and embedded in paraffin wax. Periapical lesions with the surrounding bone tissue were prepared in 5-μm sections. Immunohistochemical staining was performed by using a streptavidin-biotin method as previously described (18). Briefly,

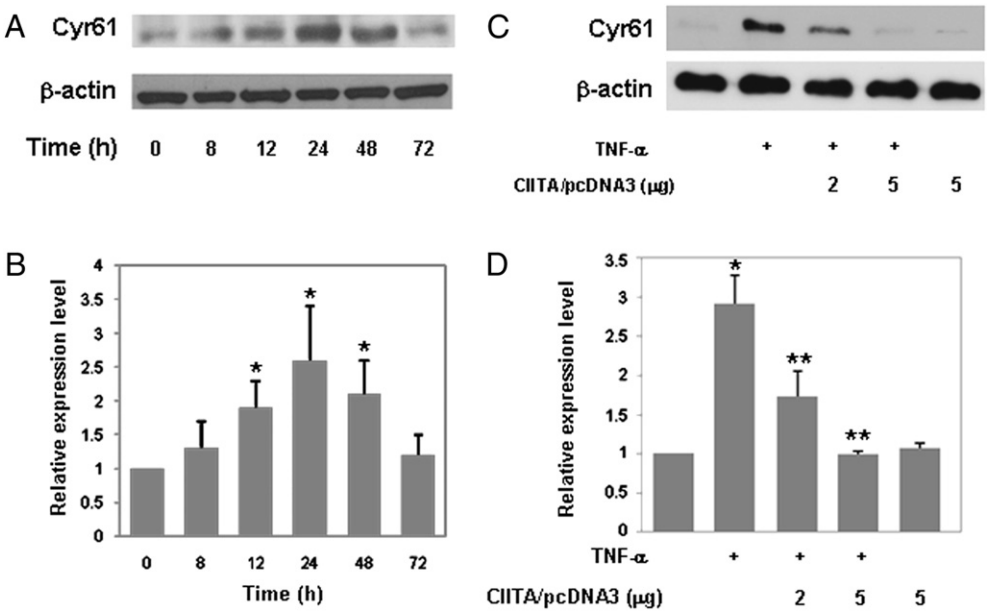

Figure 1. Synthesis of Cyr61 proteins in U2OS cells was examined by Western blot analysis. (*A*) Cells were treated with 20 ng/mL TNF-α for various incubation periods. (*B*) Data from (*A*) were quantified by densitometric analysis and expressed as the fold change relative to untreated control. Values are mean ± standard deviation of 3 independent experiments. *P < .05 versus control. (*C*) Cells with or without Myc-CIITA/pcDNA3 transfection were incubated for 24 hours with 20 ng/mL TNF-α. (*D*) Data from (*C*) were quantified by densitometric analysis and expressed as the fold change relative to untreated control. Each bar represents mean ± standard deviation of 3 independent experiments. *P < .05 versus control; **P < .05 versus TNF-α alone.