CTGF inhibits cell motility and COX-2 expression in oral cancer cells

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ABSTRACT

Oral squamous cell carcinoma (SCC) has a striking tendency to migrate and metastasize. Cyclooxygenase (COX)-2, the inducible isoform of prostaglandin synthase, has been implicated in tumor metastasis. Connective tissue growth factor (CTGF), a secreted protein that binds to integrins, modulates the invasive behavior of certain human cancer cells. However, the effect of CTGF on migration activity and COX-2 expression in human oral cells is mostly unknown. Here we found that CTGF reduced the migration and expression of COX-2 in human oral cancer cells. αvβ5 monoclonal antibody (mAb), phosphatidylinositol 3-kinase inhibitor (PI3K; Ly294002 and wortmannin) and Akt inhibitor reversed the CTGF-inhibited the migration and COX-2 down-regulation of oral cancer cells. CTGF stimulation decreased the phosphorylation of focal adhesion kinase (FAK), PI3K and Akt. In addition, c-Jun siRNA also antagonized the CTGF-inhibited migration and COX-2 expression. Moreover, CTGF decreased the binding of c-Jun to the AP-1 element on the COX-2 promoter. Taken together, our results indicated that CTGF inhibits the migration of oral cancer cells by decreasing COX-2 expression through the αvβ5 integrin receptor, FAK, PI3K, Akt, c-Jun and AP-1 signal transduction pathway.

Running title: CTGF inhibits the migration of oral cancer

Key words: CTGF; Migration; Oral cancer; FAK; Integrin

INTRODUCTION

Oral squamous cell carcinoma (SCC) represents 1–2% of all human malignancies [Banoczy, 1997; Neville and Day, 2002]. It is characterized by a high degree of local invasiveness and a high rate of metastasis to cervical lymph nodes. The migration of oral SCC into maxillary and mandibular bones is a common clinical problem [Lyons and Jones, 2007]. Because oral cancer is a type of highly malignant tumor with a potent capacity to invade locally and metastasize distantly [Greenberg et al., 2003; Thomas and Speight, 2001], an approach that decreases its ability to invade and metastasize may facilitate the development of effective adjuvant therapy.

Cyclooxygenases (COXs) are the rate-limiting enzymes that catalyze the conversion of arachidonic acid to prostaglandins (PGs). Two COX isoforms with distinct tissue distributions and physiological functions have been identified [Smith et al., 2000; Warner and Mitchell, 2004]. COX-1 is constitutively expressed in many tissues and plays important roles in the control of homeostasis [Morita, 2002]. Conversely, COX-2 is an inducible enzyme and is activated by extracellular stimuli such as growth factors and pro-inflammatory cytokines [Turini and DuBois, 2002]. Over-expression of COX-2 is frequently found in many types of cancer, including colon, lung, breast, pancreas, head, and neck cancers [Hida et al., 1998; Hwang et al., 1998; Sano et al., 1995] and is usually associated with poor prognosis and short survival. COX-2 also plays an important role in oral cancer cell migration, and COX-2 inhibitors or siRNA had also been reported to rescues the migration of oral cancer cells [Yang et al.]. Therefore, COX-2 may play a critical role in tumorigenesis, and its disruption may prevent metastasis.

Connective tissue growth factor (CTGF, also known as CCN2) belongs to the CCN family [Bork, 1993]. This family consists of six members, CTGF, NOVH, CYR61, WISP1, WISP2 and WISP3 [Perbal, 2004] that all possess an N-terminal signal peptide identifying them as secreted proteins. CCN proteins probably carry out their biological activity through binding and activating of the cell surface integrins [Perbal, 2004]. Focal adhesion kinase (FAK), a potential candidate signaling molecule,

has been shown to be capable of regulating integrin-mediated signaling [Crouch et al., 1996; Hadden and Henke, 2000]. However, the downstream signaling pathways that mediate integrin-FAK signaling are diverse, and the factors determining which pathway is used remain obscure. It has been reported that the CCN proteins involved the stimulation of cellular proliferation, migration, adhesion, extracellular matrix formation, and also the regulation of angiogenesis and tumorigenesis [Lau and Lam, 1999]. Overexpression of CTGF, WISP1, and CYR61 in tumor cells have been linked to tumor size and lymph node metastasis [Xie et al., 2001], suggesting that these CCN proteins are involved in the progression of human cancers.

Previous studies have shown that CTGF modulates cell migration and invasion in cancer cells [Chang et al., 2004; Chen et al., 2007; Tan et al., 2009]. COX-2 has been reported that modulates the cell migration and invasion of oral cancer cells [Yang et al.]. However, the effect of CTGF on COX-2 expression and migration activity in human oral cancer cells is mostly unknown. We hypothesized that CTGF might be capable of regulating oral cancer cell migration and COX-2 expression. Here we found that CTGF reduced the migration and the expression of COX-2 in human oral cancer cells. In addition, αvβ5 integrin, FAK, phosphatidylinositol 3-kinase (PI3K), Akt and AP-1 signaling pathways may be involved in the decrease of COX-2 expression and cell migration by CTGF.

MATERIALS and METHODS

Materials:

Anti-mouse and anti-rabbit IgG-conjugated horseradish peroxidase, rabbit polyclonal antibodies specific for β-actin, p85, Akt, c-Jun, β-actin and the small interfering RNAs (siRNAs) against COX-2, c-Jun and control (for experiments using targeted siRNA transfection; each consists of a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA) were purchased from Santa

Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal antibodies specific for p-FAK, p-p85 and p-Akt were purchased from Cell Signaling and Neuroscience (Danvers, MA). Rabbit polyclonal antibodies (neutralizing antibodies) specific for αvβ3(MAB1976Z), αvβ5(MAB1961Z) and α5β1(MAB1969) integrin were purchased from Chemicon (Temecula, CA). Rabbit polyclonal antibody specific for COX-2 was purchased from Cayman Chemical (Ann Arbor, MI). The recombinant human CTGF was purchased from PeproTech (Rocky Hill, NJ, USA). NS398, Ly294002, wortmannin, Akt inhibitor and curcumin were purchased from Calbiochem (San Diego, CA). Celebrex was purchased from Pharmacia Co. (Piscataway, NJ). The phosphorylation site mutant of FAK(Y397F) was a gift from Dr. J. A. Girault (Institut du Fer a` Moulin, Moulin, France). The p85α (Δp85; deletion of 35 amino acids from residues 479-513 of p85) and Akt (Akt K179A) dominant-negative mutants were gifts from Dr. W. M. Fu (National Taiwan University, Taipei, Taiwan). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

Cell Culture

The human oral cancer cell line SCC4, SAS and Cal-27 was obtained from the American Type Culture Collection (Rockville, MD). The cells were maintained in DMEM supplemented with 20 mM HEPES and 10% heat-inactivated FCS, 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37 ℃ with 5% CO_2.

Migration Assay

The migration assay was performed using Transwell (Costar, NY; pore size, 8-μm) in 24-well dishes. Before the migration assay, cells were pretreated for 30 min with different concentrations of inhibitors, including the Ly294002, wortmannin or vehicle control (0.1% DMSO). Approximately 1×10^4 cells in 100 μl of serum-free medium were placed in the upper chamber, and 300 μl of the same medium containing CTGF was placed in the lower chamber. The plates were incubated for 24

h at 37℃ in 5% CO_2, and then cells were fixed in methanol for 15 min and stained with 0.05% crystal violet in PBS for 15 min. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed with PBS. Cells on the underside of the filters were examined and counted under a microscope. Each clone was plated in triplicate in each experiment, and each experiment was repeated at least three times. The number of migrating cells in each experiment was adjusted with a cell viability assay to correct for proliferation effects of CTGF (corrected migrating cell number = counted migrating cell number/percent of viable cells) [Tang et al., 2008].

Quantitative Real-Time PCR

Total RNA was extracted from oral cancer cells using a TRIzol kit (MDBio Inc., Taipei, Taiwan). The reverse transcription reaction was performed using 2 μg of total RNA that was reverse transcribed into cDNA using oligo(dT) primer. The quantitative real time PCR (qPCR) analysis was carried out using Taqman® one-step PCR Master Mix (Applied Biosystems, CA). 2 μl of total cDNA mixtures were added per 25-μl reaction with sequence-specific primers and Taqman® probes. Sequences for all target gene primers and probes were purchased commercially (β-actin was used as internal control) (Applied Biosystems, CA). qPCR assays were carried out in triplicate (one independent RNA sample for each treatment) on an StepOnePlus sequence detection system. The cycling conditions were 10-min polymerase activation at 95 ℃ followed by 40 cycles at 95 ℃ for 15 s and 60 ℃ for 60 s. The threshold was set above the non-template control background and within the linear phase of target gene amplification to calculate the cycle number at which the transcript was detected (denoted C_T).

Western Blot Analysis

Cellular lysates were prepared as described [Tang et al., 2008]. Proteins were resolved on SDS-PAGE and transferred to Immobilon polyvinyldifluoride (PVDF)

membranes. The blots were blocked with 4% BSA for 1 hr at room temperature and then probed with rabbit anti-human antibodies against FAK, p-FAK, p85 or p-p85 (1:1000) for 1 hr at room temperature. After three washes, the blots were subsequently incubated with a donkey anti-rabbit peroxidase-conjugated secondary antibody (1:1000) for 1 hr at room temperature. The blots were visualized with enhanced chemiluminescence and Kodak X-OMAT LS film (Eastman Kodak, Rochester, NY).

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation analysis was performed as described [Huang and Chen, 2005]. DNA immunoprecipitated with anti-c-Jun was purified and extracted with phenol-chloroform. The purified DNA pellet was subjected to PCR, and PCR products were resolved with 1.5% agarose gel electrophoresis and visualized with UV light. The primers 5'-TAAGGGGAGAGGAGGGAAAAAT-3' and 5'-ACAATTGTCGCTAACCGAG-3' were utilized to amplify across the COX-2 promoter region (–119 to –14) [Chang et al., 2005].

Statistics

The values given are means ± S.E.M. Statistical analysis between two samples was performed using the Student's *t* test. Statistical comparisons involving more than two groups were performed using one-way analysis of variance (ANOVA) with Bonferroni's *post hoc* test. In all cases, $p < 0.05$ was considered to be significant.

RESULTS

CTGF reduced migration and COX-2 expression in oral cancer cells

CTGF has been reported regulates migration and invasion of human cancer cells [Chang et al., 2004; Chen et al., 2007; Tan et al., 2009]. The CTGF for oral cancer cell migration was examined using the Transwell assay. Treatment of SCC4 cells with

CTGF (5-50 ng/ml) reduced cell migration (Fig. 1A). In addition, CTGF also dose-dependently reduced other human oral cancer cell migration (SAS and Cal-27 cells) (Fig. 1B). However, treatment of these oral cancer cells with CTGF did not affect cell viability by DAPI staining and MTT assay (data not shown). Previous study has shown that COX-2 mediated cell motility in human oral cancer cells [Yang et al.]. We therefore, hypothesized that COX-2 may be involved in CTGF-mediated oral cancer migration. Treatment of cells with CTGF reduced mRNA and protein expression of COX-2 (Fig. 1C&D). To confirm COX-2 mediated CTGF-reduced cell migration, the COX-2 specific inhibitors (Celebrex and NS-398) were used. Celebrex and NS-398 reversed CTGF-mediated cell migration (Fig. 1E). When the SCC4 cells were transfected with COX-2 or control siRNA for 24 hr, the Western blot analysis showed that the expression of protein levels of COX-2 was suppressed by transfection with COX-2 siRNA (Fig. 1F; upper panel). Transfection of cells with COX-2 siRNA reversed the CTGF- reduced cell migration (Fig. 1F; lower panel). We also examined human oral cancer tissues for expression of CTGF and COX-2 using Western blot analysis. We found that oral cancer tissues expressed CTGF and COX-2 (Fig. 1G). These data suggest that CTGF-reduced cancer migration may occur via down-regulation of the COX-2.

CTGF reduced oral cancer cell migration through αvβ5 integrin

Previous study has shown CTGF affects cell migration through integrin receptor signaling [Chen et al., 2007; Tan et al., 2009]. We therefore hypothesized that integrin receptor signaling pathway may be involved in CTGF-mediated oral cancer cell migration. Pretreatment of cells for 30 min with anti-αvβ5 but not anti-αvβ3 and α5β1 monoclonal antibody (mAb) markedly reversed the CTGF-reduced cancer migration (Fig. 2A). In addition, treatment of cells with αvβ5 but not αvβ3 and α5β1 mAb also reversed the CTGF-reduced COX-2 expression (Fig. 2B). These results indicate that CTGF reduced cell migration in human oral cancer cells via αvβ5 integrin receptor.

The FAK, PI3K and Akt signaling pathways are involved in the CTGF-reduced migration of oral cancer cells

FAK has been shown to be capable of regulating integrin-mediated signaling [Mitra and Schlaepfer, 2006; Schlaepfer and Hunter, 1998]. Phosphorylation of tyrosine 397 of FAK has been used as a marker of FAK activity. As shown in Fig. 2C, FAK phosphorylation decreased in a time-dependent manner in response to CTGF stimulation. Transfection of cells with FAK(Y397F) mutant reversed the CTGF-reduced cell migration and COX-2 expression (Fig. 2D&E). Phosphorylation of tyrosine 397 of FAK may provide a binding site for the Src homology 2 domain of the p85 subunit of PI3K [Chan et al., 1999]. We then examined whether CTGF stimulation also reduces the association of FAK with PI3K. Treatment of oral cancer cells with CTGF led to a significant decrease of phosphorylation of p85 subunit of PI3K (Fig. 3A). To explore whether PI3K is involved in CTGF-reduced cell migration, PI3K inhibitor Ly294002 and wortmannin were used. As shown in Fig. 3B,C,E, pretreatment of SCC4 cells with Ly294002 and wortmannin reversed CTGF-inhibited migration activity and COX-2 expression of oral cancer cells. We then directly measured the Akt phosphorylation in response to CTGF stimulation. Figure 3A shows that CTGF decreased Akt phosphorylation (serine 473) in a time-dependent manner. Furthermore, Akt inhibitor also antagonized CTGF-inhibited cell migration and COX-2 expression (Fig. 3B,D,E). Transfection of cells with p85 and Akt mutant also antagonized the potentiating effect of CTGF (Fig. 3F&G). Taken together, these results indicate that the αvβ5 integrin/FAK/PI3K and Akt pathway is involved in CTGF-inhibited migration activity and COX-2 expression of human oral cancer cells.

Involvement of AP-1 in CTGF-inhibited cell migration and COX-2 expression

The promoter region of human COX-2 contains AP-1, NF-κB, CCAAT/enhancer-binding protein and SP binding sites [van de Stolpe and van der Saag, 1996]. AP-1 plays a critical role in COX-2 expression [Lu et al.]. To examine

the role of the AP-1 binding site in CTGF-mediated migration and COX-2 expression, an AP-1 inhibitor (curcumin) was used. Pretreatment of cells with curcumin reversed CTGF-reduced cell migration and COX-2 expression (Fig. 4A-C). It has been reported that the AP-1 binding site between –67 and –61 was important for the activation of the COX-2 gene [Iniguez et al., 2000]. AP-1 activation was further evaluated by analyzing the chromatin immunoprecipitation assay. *In vivo* dis-binding of c-Jun to the AP-1 element of the COX-2 promoter occurred after CTGF stimulation (Fig. 5A). Dis-binding of c-Jun to the AP-1 element by CTGF was reversed by Ly294002 and Akt inhibitor (Fig. 5A). Transfection of cells with c-Jun siRNA suppressed the expression of c-Jun (Fig. 5B). CTGF-inhibited cell migration and COX-2 expression was also reversed by c-Jun siRNA but not by control siRNA (Fig. 5C&D). Taken together, these data suggest that activation of the FAK, PI3K, Akt, c-Jun, and AP-1 pathways are required for the CTGF-inhibited cell migration and COX-2 expression in human oral cancer cells.

DISCUSSION

The elucidation of the molecular biology of cancer cells in recent years has identified various molecular pathways that are altered in different cancers. This information is currently being exploited to develop potential therapies that target molecules in these pathways. To achieve metastasis, cancer cells must evade multiple barriers and overcome certain rules. Several discrete steps are discernible in the biological cascade leading to metastasis: loss of cellular adhesion, increased motility and invasiveness, entry and survival into the circulation, entrance into new tissue, and eventual colonization of a distant site [Gupta and Massague, 2006]. The mechanism of metastasis is a complicated and multistage process, however our study showed that CTGF inhibits cell migration and the expression of COX-2 in human oral cancer cells. Here, we provide evidence that COX-2 acts as crucial transducers of cell signaling,

regulating cell migration and CTGF acts as a critical mediator of the metastasis activity of cancer cells in the tumor microenvironment.

COX-2 is a pleiotropic enzyme that mediates many physiological functions such as inhibition of cell apoptosis, augmentation of angiogenesis, and increased cell motility. These COX-2–mediated functions are regulated in part by various proteins such as B-cell lymphoma [Sun et al., 2002], myeloid cell leukemia-1, VEGF-A [Ma et al., 2002] and metalloproteinases [Dohadwala et al., 2002]. It has been reported that the expression of COX-2 is associated with a metastatic phenotype of oral cancer cells [Yang et al.]. In this study, we found that CTGF inhibited COX-2 expression in human oral cancer cells. On the other hand, COX-2 inhibitors antagonized CTGF-reduced cell motility. In addition, the inhibition of CTGF-inhibited COX-2 protein expression with siRNA significantly reversed CTGF-inhibited migration. However, COX-2 inhibitors and siRNA did not affect the basal migration activity of oral cancer cells (Supplemental data Fig. 1S). Therefore, COX-2 may be the CTGF-responsive mediator, and lead to decrease cancer migration and metastasis.

FAK, a potential candidate signaling molecule, has been shown to be capable of regulating integrin-mediated signaling [Miranti and Brugge, 2002]. We demonstrate that CTGF decreased phosphorylation of tyrosine 397 of FAK. Furthermore, the FAK(Y397F) mutant antagonized the CTGF-inhibited migration activity and COX-2 expression, suggesting that FAK inhibition is an obligatory event in CTGF-reduced migration in these cells. FAK contains tyrosine residues in motifs for binding to SH2 domain. Phosphorylated tyrosine 397 of FAK has been shown to serve as a binding site for the SH2 domain of the p85 subunit of PI3K [Chen et al., 1996]. Phosphorylation of the p85 subunit is required for activation of the p110 catalytic subunit of PI3K. Pretreatment of oral cancer cells with PI3K inhibitors Ly294002 and wortmannin antagonized the decrease of chemomigration by CTGF. The cytoplasmic serine kinase Akt was found to be reduced by CTGF stimulation in oral cancer cells. In addition, treatment of cells with Akt inhibitor also reversed CTGF-inhibited cell migration and COX-2 expression. This was further confirmed by the result that the

dominant-negative mutant of p85 and Akt reversed the migration and COX-2 expression by CTGF stimulation. Take together, our results provide evidence that CTGF reduces migration and COX-2 expression in oral cancer cells through FAK/PI3K/Akt signaling pathway.

It has been reported that chemokines induced COX-2 secretion through AP-1 dependent pathway [Chien et al., 2009; Lu et al.]. The AP-1 sequence binds to members of the Jun and Fos families of transcription factors. These nuclear proteins interact with the AP-1 site as Jun homodimers or Jun-Fos heterodimers formed by protein dimerization through their leucine zipper motifs. The results of this study show that CTGF reduced cell migration and COX-2 expression was reversed by c-Jun siRNA. Furthermore, CTGF decreased the binding of c-Jun to the AP-1 element on COX-2 promoter, as shown by ChIP assay. The dis-binding of c-Jun to the AP-1 element was also reversed by Ly294002 and Akt inhibitor. These results indicate that CTGF might act through the αvβ5 integrin, FAK, PI3K, Akt, c-Jun and AP-1 pathway to reduce cell migration and COX-2 expression in human oral cancer cells. In addition, Ly294002, wortmannin, Akt inhibitor and curcumin or FAK mutant also reversed CTGF-inhibited cell migration in other oral cancer cells (SAS and Cal-27 cells) (Supplemental data Fig. 2S). Therefore, the same signaling pathways are involved in all oral cancer cells.

In conclusion, we present here a novel mechanism of CTGF-reduced migration of human oral cancer cells by down-regulation of COX-2. CTGF inhibits COX-2 expression by binding to the αvβ5 integrin receptor and reduction of FAK, PI3K, Akt which inhibits binding of c-Jun to AP-1 site, resulting in the reduction tumor migration.

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FIGURE LEGENDS

Fig. 1 CTGF reduced the migration activity of human oral cancer cells

(A&B) Cells were incubated with various concentrations of CTGF, and *in vitro* migration activities measured with the Transwell after 24 hr. (C) SCC4 cells were incubated with CTGF (50 ng/ml) for indicated time intervals, and COX-2 expression was examined by western blot analysis. (D) SCC4 cells were incubated with CTGF (50 ng/ml) for 24 hr, and the mRNA expression of COX-2 was examined by qPCR. (E) SCC4 cells were pretreated with celebrex (10 μM) or NS-398 (20 μM) for 30 min followed by stimulation with CTGF. The *in vitro* migration activity measured with the Transwell after 24 hr. SCC4 cells were transfected with COX-2 or control siRNA for 24 hr, the COX-2 expression was examined using western blot analysis (F; upper panel). Cells were transfected with COX-2 or control siRNA for 24 hr followed by stimulation with CTGF, and *in vitro* migration was measured with the Transwell after 24 hr (F; lower panel). (G) Total protein were extracted from human oral cancer tissues and subjected to Western blot analysis for CTGF and COX-2. Results are expressed as the mean ± S.E. *, $p < 0.05$ compared with control. #, $p < 0.05$ compared with CTGF-treated group.

Fig. 2 CTGF reduced oral cancer cell migration through αvβ5 integrin.

(A&B) SCC4 cells were pretreated with αvβ3, αvβ5 and αvβ1 monoclonal antibody (10 μg/ml) for 30 min followed by stimulation with CTGF. The *in vitro* migration activity and COX-2 expression were measured with Transwell and western blotting analysis. (C) SCC4 cells were incubated with CTGF for indicated time intervals, and p-FAK expression was determined by western blot analysis. (D&E) Cells were transfected with FAK mutant or control vector for 24 hr followed by stimulation with CTGF, and *in vitro* migration and COX-2 expression were measured with the Transwell and

western blotting analysis. Results are expressed as the mean ± S.E. *, $p < 0.05$ compared with control. #, $p < 0.05$ compared with CTGF-treated group.

Fig. 3 PI3K/Akt pathway is involved in CTGF-mediated migration in human oral cancer cells.

(A) SCC4 cells were incubated with CTGF for indicated time intervals, and p-PI3K and p-Akt expression was determined by western blot analysis. (B-E) SCC4 cells were pretreated with Ly294002 (10 μM), wortmannin (10 μM) or Akt inhibitor (10 μM) for 30 min followed by stimulation with CTGF. The *in vitro* migration activity and COX-2 expression were measured with Transwell, western blotting analysis and qPCR. (F&G) Cells were transfected with p85 and Akt mutant or control vector for 24 hr followed by stimulation with CTGF, and *in vitro* migration and COX-2 expression were measured with the Transwell and western blotting analysis. Results are expressed as the mean ± S.E. *, $p < 0.05$ compared with control. #, $p < 0.05$ compared with CTGF-treated group.

Fig. 4 AP-1 is involved in CTGF-mediated migration in human oral cancer cells.

(A-C) SCC4 cells were pretreated with curcumin (10 μM) for 30 min followed by stimulation with CTGF. The *in vitro* migration activity and COX-2 expression were measured with Transwell, western blotting analysis and qPCR. Results are expressed as the mean ± S.E. *, $p < 0.05$ compared with control. #, $p < 0.05$ compared with CTGF-treated group.

Fig. 5 FAK/PI3K/Akt pathway is mediated CTGF-reduced AP-1 activation.

(A) Cells were pretreated with Ly294002, and Akt inhibitor then stimulated with CTGF for 120 min, and the chromatin immunoprecipitation assay was then performed. Chromatin was immunoprecipitated with anti-c-Jun. One percentage of the precipitated chromatin was assayed to verify equal loading

(input). (B) SCC4 cells were transfected with c-Jun or control siRNA for 24 hr, the c-Jun expression was examined using western blot analysis. (C&D) Cells were transfected with c-Jun or control siRNA for 24 hr followed by stimulation with CTGF, and *in vitro* migration and COX-2 expression were measured with the Transwell and western blotting analysis. Results are expressed as the mean ± S.E. *, $p < 0.05$ compared with control. #, $p < 0.05$ compared with CTGF-treated group.

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