Biochemical and Biophysical Research Communications 446 (2014) 487–492

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Interleukin-1α promotes extracellular shedding of syndecan-2 via induction of matrix metalloproteinase-7 expression

Mi-Jung Kwon [a], Eunkyoung Hong [a], Youngsil Choi [a], Duk-Hee Kang [b,*], Eok-Soo Oh [a,*]

[a] Department of Life Sciences, Division of Molecular Life Sciences and Center for Cell Signaling Research, Ewha Womans University, Seoul 120-725, Republic of Korea
[b] Division of Nephrology, Department of Internal Medicine, Ewha Womans University School of Medicine, Ewha Medical Research Center, Seoul, Republic of Korea

ARTICLE INFO

*Article history:*
Received 17 February 2014
Available online 12 March 2014

*Keywords:*
Interleukin
Syndecan
Matrix metalloproteinase
Extracellular matrix

ABSTRACT

The cell surface heparan sulfate proteoglycan, syndecan-2, is known to play an important role in the tumorigenic activity of colon cancer cells. In addition, the extracellular domain of syndecan-2 is cleaved by matrix metalloproteinase-7 (MMP-7) in various colon cancer cells, but factors involved in regulating this process remain unknown. Here, we demonstrate a role for interleukin-1α (IL-1α) in syndecan-2 shedding in colon cancer cells. Treatment of low metastatic (HT-29) and highly metastatic (HCT-116) colon cancer cells with various soluble growth factors and cytokines revealed that IL-1α specifically increased extracellular shedding of syndecan-2 in a concentration- and time-dependent manner. IL-1α did not affect the expression of syndecan-2, but did significantly reduce its cell surface levels. Notably, IL-1α increased the mRNA expression and subsequent secreted levels of MMP-7 protein and enhanced the phosphorylation of p38 and ERK mitogen-activated protein kinases. Furthermore, increased syndecan-2 shedding was dependent on the mitogen-activated protein kinase-mediated MMP-7 expression. Taken together, these data suggest that IL-1α regulates extracellular domain shedding of syndecan-2 through regulation of the MAP kinase-mediated MMP-7 expression in colon cancer cells.



## 1. Introduction

Syndecans are a family of cell surface heparin sulfate proteoglycans known to play critical roles in regulating multiple cellular functions, including those related to cancer biology. For instance, knockdown of syndecan-1 using small interfering RNA (shRNA) promotes apoptosis of myeloma cells and dramatically diminishes tumor cell growth [1] and vasculogenesis in mouse models [2]. Consistent with this, overexpression of syndecan-1 has been shown to induce approximately a 2-fold increase in the proliferation of endometrial cancer cells [3], and syndecan-1 mRNA levels are up-regulated in pancreatic cancer in association with accelerated tumor growth [4]. Similarly, syndecan-2 expression is increased in several cancer cell lines, including colon adenocarcinoma, melanoma and fibrosarcoma, an up-regulation that is critical for cancer cell behaviors such as migration [5–7]. Therefore, syndecans could be expected to play critical roles as adhesion receptors in cancer progression. Interestingly, the presence of a dibasic peptide sequence adjacent to the plasma membrane in all syndecans predicts that the extracellular domain of syndecans could be cleaved by extracellular proteases, a process known as extracellular shedding [8]. Indeed, syndecan shedding has been demonstrated in various settings, including colon cancer cells [9].

Colon cancer, one of the most common cancers in the world, is known for its poor prognosis because of its high propensity for metastasis. During colon cancer carcinogenesis, up-regulated syndecan-2 influences colon cancer cell behavior. Syndecan-2 may modulate tumorigenic activity by regulating adhesion to the extracellular matrix (ECM) or by regulating the activity and localization of matrix metalloprotease-7 (MMP-7) [10]. The functions of syndecan-2 can be further extended through extracellular shedding. We have shown that, in colon cancer cells, MMP-7, which is an important regulator of colon cancer cell migration and invasion, mediates extracellular shedding of syndecan-2 [10]. Therefore, any

*Abbreviations:* AP-1, activating protein-1; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IFN-γ, interferon-gamma; IL-1, interleukin-1; IL-R, interleukin receptor; IRAK, interleukin-1 receptor-associated kinases; MAP kinase, mitogen-activated protein kinase; MMP, matrix metalloprotease; NF-κB, nuclear factor-κB; shRNA, small interfering RNA.

* Corresponding authors. Address: Division of Nephrology, Ewha University School of Medicine, 911 Mok-dong Yangchun-ku, Seoul 158-710, Republic of Korea. Fax: +82 2 2655 2076 (D.-H. Kang). Address: Department of Life Sciences, Ewha Womans University, 52, Ewhayeodae-gil, Seodaemoon-Gu, Seoul 120-750, Republic of Korea. Fax: +82 2 3277 3760 (E.-S. Oh).

*E-mail addresses:* dhkang@ewha.ac.kr (D.-H. Kang), OhES@ewha.ac.kr (E.-S. Oh).

http://dx.doi.org/10.1016/j.bbrc.2014.02.142
0006-291X/

regulators that mediate shedding of syndecan-2 extracellular domains might participate in the regulation of colon cancer progression.

Interleukin-1 (IL-1) is a pleiotropic cytokine that primarily affects inflammatory and immune responses, but also regulates other homeostatic functions of the body [11]. A variety of cell types, including lymphocytes, monocytes, fibroblasts, endothelial cells and epithelial cells, can produce IL-1 [12,13]. The pro-inflammatory activities of IL-1α are mainly due to stimulation of the expression of genes encoding inflammatory mediators [14]. Importantly, elevated expression of IL-1 in cancers such as colon, melanoma, and breast cancer [11] regulates tumor progression by inducing the expression of metastatic and angiogenic genes and growth factors, such as MMPs, vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) [15]. Matsuo et al. showed that IL-1α is secreted by colon cancer cells and influences tumor invasiveness and angiogenesis by inducing the expression of cancer-related genes [16]. In general, IL-1α transduces intracellular signals through its type 1 receptor (IL-1R1). Subsequently, a complex is formed containing IL-1R-associated kinases (IRAK1 and IRAK2), Toll-interacting protein (TOLLIP), and the adapter protein (MYD88). IRAK is rapidly phosphorylated and associates with TNF receptor-associated factor 6 (TRAF6). This association is necessary for downstream IL-1-induced activation of MAP kinase signaling and nuclear factor-κB (NF-κB), which are crucial mediators of the expression of inflammatory cytokines, including IL-6 and IL-8 [17].

Since the inflammatory response is well known to enhance the progression of cancer, and extracellular shedding of syndecan commonly occurs during inflammatory responses [18–21], we investigated whether inflammatory cytokines are involved in the regulation of syndecan-2 shedding. In this study, we show that, among various cytokines and growth factors, IL-1α specifically enhances shedding of syndecan-2 by enhancing MMP-7 expression.

## 2. Materials and methods

### 2.1. Materials and antibodies

Monoclonal (mAb) antibody to synecan-2 was produced by AdipoGen (Korea) [7]. mAbs to phospho-extracellular-signal-regulated kinase (ERK) and ERK were purchased from Santa Cruz (CA, USA) and mAbs to phospho-p38 and p38 were purchased from Cell signaling (MA, USA). mAbs to MMP-7 was purchased from Abcam (Cambridge, England). SB239063 was purchased from Calbiochem (Darmstadt, Germany) and PD98059 was purchased from Sigma Aldrich (MO, USA). IL-1α, IL-6, TNFα, and EGF were purchased from R&D Systems (MN, USA). GM6001 and FGF-2 were purchased from Millipore (MA, USA). Polyclonal antibody to NF-κB p65 and Bay 11-7085 were purchased from Santa Cruz (CA, USA).

### 2.2. Cell culture

Human colon adenocarcinoma cell line HT-29 and HCT-116 cells were maintained in Mccoy's 5A complete media (Welgene, Korea) supplemented with 10% (v/v) fetal bovine serum (FBS, Hyclone) with gentamycin (50 g/ml, Sigma) at 37 °C in 5% $CO_2$ in a humidified atmosphere.

### 2.3. RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA extracted from treated cells was used as template for reverse transcriptase reaction. Aliquots of cDNA were amplified using the following primers: human MMP-7 (forward) 5′-GGTCACC TACAGGATCGTATCATAT-3′ and (backward) 5′-CATCACTGCATTA GGATCAGAGGAA-3′, human GAPDH (forward) 5′-CCACCCATGGCA AATTCCATGGCA-3′ and (backward) 5′-TCTAGACGGCAGGTCAGGT CCACC-3′, human MMP-2 (forward) 5′-CAAGGACCGGTTTATTTG GC-3′ and (backward) 5′-ATTCCCTGCGAAGAACACAGC-3′, human MMP-9 (forward) 5′-TTGACAGCGACAAGAAGTGG-3′ and (backward) 5′-GCCATTCACGTCGTCCTTAT-3′, human SDC2 (forward) 5′-ACATCTCCCCTTTGCTAACGGC-3′ and (backward) 5′-TAACTCCAT CTCCTTCCCCAGG-3′, human β-actin (forward) 5′-TGGAATCCTGTG GCATCCATGAAA-3′ and (backward) 5′-TAAAACGCAGCTCAGTAAC AGTCCG-3′. After an initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for human MMP-7, human β-actin and GAPDH, 52 °C for human MMP-2, MMP-9 and human SDC2 for 60 s, and extension at 72 °C for 60 s. The reaction products were analyzed in 1% agarose gels.

### 2.4. Immunoblotting

The cultures were washed twice with phosphate-buffered-saline (PBS) and the cells were lysed in RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 2 mM $Na_3VO_4$) containing a protease inhibitor cocktail (1 mg/ml aprotitin, 1 mg/ml antipain, 5 mg/ml leupeptin, 1 mg/ml pepstatin A, 20 mg/ml phenylmethylsulfonyl fluoride). The lysates were clarified by centrifugation at 13,000×*g* for 15 min at 4 °C, denatured with SDS sample buffer, boiled, and analyzed by SDS–PAGE. The proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore) and probed with appropriate antibodies, followed by species-specific horseradish peroxidase-conjugated secondary antibodies (Amersham Life science). The signals were detected by enhanced chemiluminescence (ECL; AbClon).

### 2.5. Slot blotting

Conditioned media were slot-blotted onto a nitrocellulose membrane in a Bio-Rad apparatus (Bio-Rad, Hercules, CA, USA). Before blotting, the membrane was stained with Ponceau S. The membrane was then washed in 0.05% Tween-20 in Tris-buffered saline (TBS: 50 mM Tris–HCl, pH 7.4, 150 mM NaCl), blocked in TBS-T with 5% milk, washed, and probed with anti-syndecan-2 monoclonal antibody for overnight at 4 °C, followed by species-specific horseradish peroxidase conjugated secondary antibodies from Amersham Life science (West Grove, Pennsylvania). The signals were detected by enhanced chemiluminescence (ECL; Anigen, Korea).

### 2.6. Cell proliferation assay

Cell proliferation was measured by a colorimetric assay using 3-(4,5-dimethythiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT). In brief, cells were harvested with 0.05% trypsin/EDTA and seeded into 96 well plates at $5 \times 10^3$ cells/well. After incubation, the medium containing 0.5 mg/ml MTT (Sigma) was added to each plate in a volume of 100 μl and cells were incubated for 1 h. The medium was then removed and 100 μl of acidic isopropanol (90% isopropanol, 0.5% SDS, 25 mM NaCl) was added to each plate. The mean concentration of absorbance at 570 nm in each set of all samples was measured using a 96-well microtiter plate reader (Dynatech, Chantilly, VA).

### 2.7. Luciferase assay

HCT-116 cells were plated in 24-well plates 24 h prior to transfection. Cells were then co-transfected with 0.8 μg of pGL3-basic vector or MMP-7 reporter constructs (a generous gift from Dr. Seung Teak Lee at Yonsei University in Korea), together with

0.08 μg of pCMV/β-galactosidase as the normalizing control using Vivamagic transfection reagent (Vivagen, Korea). 24 h later from transfection, cells were treated with IL-1α for the indicated periods of time, washed with PBS and lysed with reporter lysis buffer in luciferase kit (Promega, USA). Soluble extracts were harvested and assayed for both luciferase and β-galactosidase activities according to the manufacturer's instruction. The relative luciferase unit (RLU, luciferase activity/β-galactosidase activity) was calculated to normalize the luciferase values for transfection efficiency.

### 2.8. Immunofluorescence

Cells were seeded onto glass coverslips in 12-well plates, incubated for 24 h, and then fixed with 3.5% paraformaldehyde in PBS at room temperature for 10 min. After rinsing three times with PBS, cells were permeabilized with 0.5% Triton X-100 in PBS at room temperature for 5 min and blocked with 0.5% BSA in PBS for 1 h. After being washed, cells were stained with the appropriate primary antibody for overnight at 4 °C. After being washed with PBS, cells were incubated with Texas red-conjugated rabbit antibody for 1 h at room temperature. Coverslips were washed with PBS and then mounted on glass slides using mounting solution containing DAPI. Images were obtained by digital imaging florescence microscopy using a CCD camera (Carl Zeiss).

### 2.9. Fluorescence-activated cell sorting (FACS)

HT-29 cells were washed with PBS and released by the addition of 1 mM EDTA, followed by the addition of PBS. After pelleting, cells were resuspended in PBS and incubated anti-syndecan-2 antibody in 10% FBS in PBS for 1 h on 4 °C followed by PBS containing 0.05% Tween-20, washed three times and incubated FITC-conjugated anti-mouse IgG in 10% FBS in PBS for 1 h. Syndecan-2 expressions were analyzed by flow cytometry.

### 2.10. Statistical analysis

Data are represented as the mean from three independent experiments. Statistical analysis was performed using an unpaired Student's *t* test. A *p* value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. IL-1α promotes extracellular shedding of syndecan-2 in colon cancer cells

To investigate soluble factors involved in regulating the extracellular shedding of syndecan-2 in colon cancer, we treated both low metastatic HT-29 and highly metastatic HCT-116 colon cancer cells with the growth factors, FGF-2 and EGF, and cytokines IL-1α, IL-6, and TNF-α, and determined the presence of soluble forms of shed syndecan-2 in the conditioned media using slot blotting with an anti-syndecan-2 antibody. At 5 h, shed syndecan-2 levels in conditioned media from HT-29 and HCT-116 cells were increased by treatment with IL-1α, and IL-1α-increased shed syndecan-2 levels became more evident at 24 h after treatment (Fig. 1A). On the other hand, we could not detect significant increase of shed syndecans-2 levels by treatment with any other growth factors or cytokines (Fig. 1A). These observations suggest the possible involvement of IL-1α in syndecan-2 shedding in colon cancer cells, consistent with the known importance of IL-1α in colon cancer progression [16]. To extend these observations, we examined the effects of different IL-1α concentrations and duration of exposure on syndecan-2 extracellular shedding in colon cancer cells. As shown in Fig. 1B, IL-1α promoted syndecan-2 shedding in a time- and concentration-dependent manner. In contrast, IL-1α treatment did not affect syndecan-2 mRNA expression (Fig. 1C) or proliferation of HT-29 and HCT-116 colon cancer cells (Fig. 1D), suggesting that the increase in shed syndecan-2 in the conditioned media was not due to increased cell numbers or increased syndecan-2 expression. In addition, we examined the effect of extracellular shedding of syndecan-2 at the cell surface by stably expressing syndecan-2 in HT-29 cells, which express very low basal levels of syndecan-2, and then treated cells with IL-1α. FACS analyses revealed that IL-1α reduced the cell surface levels of syndecan-2 (Fig. 1E). Taken together, these data suggest that IL-1α specifically promotes extracellular shedding of syndecan-2 in colon cancer cells.

### 3.2. IL-1α regulates the expression of MMP-7

Previously, we reported that interaction with the extracellular domain of syndecan-2 activates MMP-7 in colon cancer cells, and MMP-7 thus activated promotes shedding of syndecan-2 [10]. Therefore, we investigated whether the increase in shed syndecan-2 induced by IL-1α was linked to MMP-7 expression. Among growth factors and cytokines examined (Fig. 1A), IL-1α strongly up-regulated MMP-7 mRNA expression (Fig. 2A). This increase in MMP-7 mRNA expression was reached a maximum level 5 h after treatment, but other factors tested showed no significant effect on MMP-7 mRNA expression (Fig. 2A). Consistent with this, IL-1α robustly increased the levels of MMP-7 protein in HT-29 cell conditioned media (Fig. 2B). The IL-1α-induced enhancement of both MMP-7 mRNA and protein levels was time and concentration dependent (Fig. 2C). Consistently, the luciferase reporter assay in HCT-116 cells revealed that IL-1α enhanced transcription of MMP-7 gene (Fig. 2D). By comparison, the basal expression levels of MMP-2 and -9 remained unchanged in response to IL-1α treatment (Fig. 2E). Collectively, these data suggest that IL-1α specifically induces the expression of MMP-7.

### 3.3. IL-1α promotes MMP-7 expression through the MAP kinase signaling pathway

It is well known that IL-1α is involved in the MAP kinase signaling pathway, and this pathway is responsible for expression of metastatic genes like MMPs [14,16,22]. We thus next investigated whether IL-1α was involved in the regulation of the MAP kinase signaling pathway in colon cancer cells. IL-1α treatment significantly increased the phosphorylation of both p38 and ERK in HT-29 compared with untreated control cells, an effect that was evident within 0.5 h (Fig. 3A). Moreover, pretreatment with the specific p38 inhibitor SB239063 or MEK inhibitor PD98059 significantly reduced MMP-7 mRNA expression (Fig. 3B) and eliminated the increased in MMP-7 protein levels in conditioned media in response to IL-1α treatment (Fig. 3C). Collectively, these observations suggest that IL-1α regulates MMP-7 expression through the MAP kinase signaling pathway.

Having shown that IL-1α enhanced the extracellular shedding of syndecan-2 (Fig. 1) and increased the expression of MMP-7 (Fig. 2), we next investigated whether MAP kinase-mediated MMP-7 expression is involved in regulating the extracellular shedding of syndecan-2. As expected, IL-1α increased the expression of MMP-7 and the levels of shed syndecan-2 in conditioned media; notably, both effects were reduced by inhibition of p38 and ERK activation (Fig. 3C). In addition, GM6001, a broad-spectrum MMP inhibitor, completely blocked IL-1α-induced shedding of the syndecan-2 extracellular domain (Fig. 3D). Taken together, these data suggest that IL-1α enhances extracellular shedding of syndecan-2 via MAP kinase-mediated regulation of MMP-7 expression.

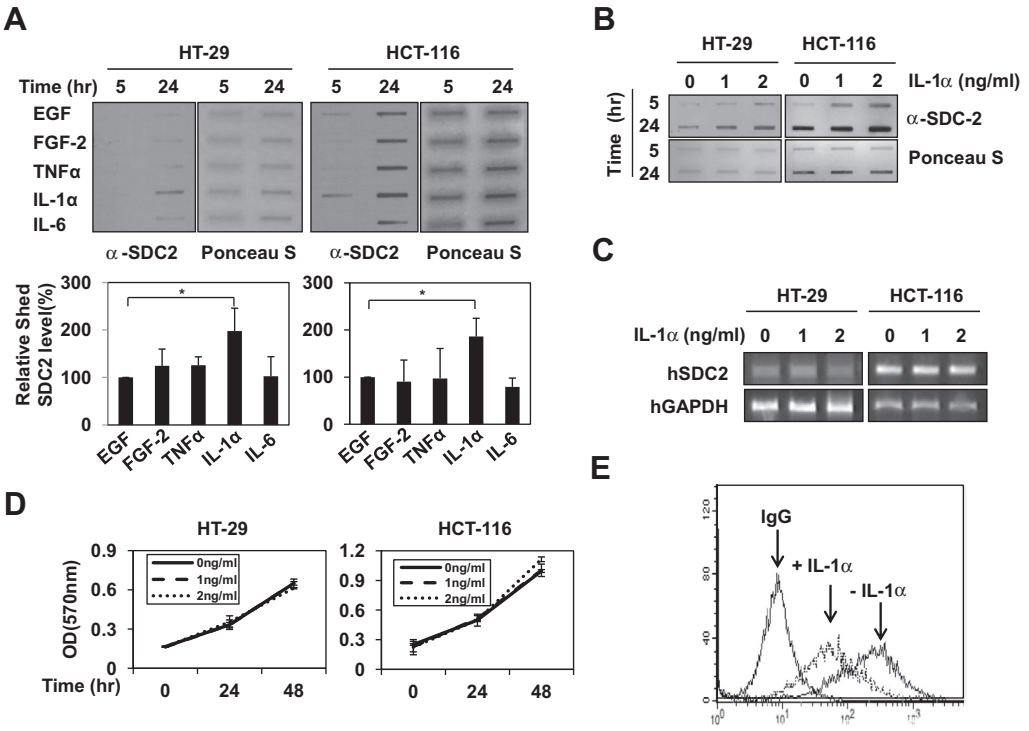


**Fig. 1.** IL-1α promotes extracellular shedding of syndecan-2 in colon cancer cells. (A) *Top panel:* After starving in serum-free media overnight, HT-29 and HCT-116 cells were treated with the indicated soluble factors. Conditioned media were collected and analyzed by slot blotting with an anti-syndecan-2 antibody (α-SDC2). Protein loading in blots was determined by Ponceau S staining. *Bottom panel:* Quantitative analysis of shed syndecan-2 levels in conditioned media were performed using Image J program. The level of shed syndecan-2 in conditioned media from cells treated with EGF was used as a control. The means of shed syndecan-2 at three independent experiments ± SEM are shown; $^{*}p < 0.05$. (B) Cells were treated with the indicated amount of IL-1α for the indicated periods of time. Conditioned media were analyzed by slot blotting with an anti-syndecan-2 antibody. (C) Cells were treated with 1 ng/ml of IL-1α for 24 h. Total RNA was extracted, and expression of syndecan-2 was analyzed by RT-PCR. (D) Cells were treated with the indicated amount of IL-1α, and cell numbers at the indicated times were evaluated by MTT assay. Experiments were performed in duplicate, with triplicate samples. (E) HT-29 cells stably expressing syndecan-2 were treated with 1 ng/ml of IL-1α for 24 h. Cell surface expression of syndecan-2 was analyzed by flow cytometry.

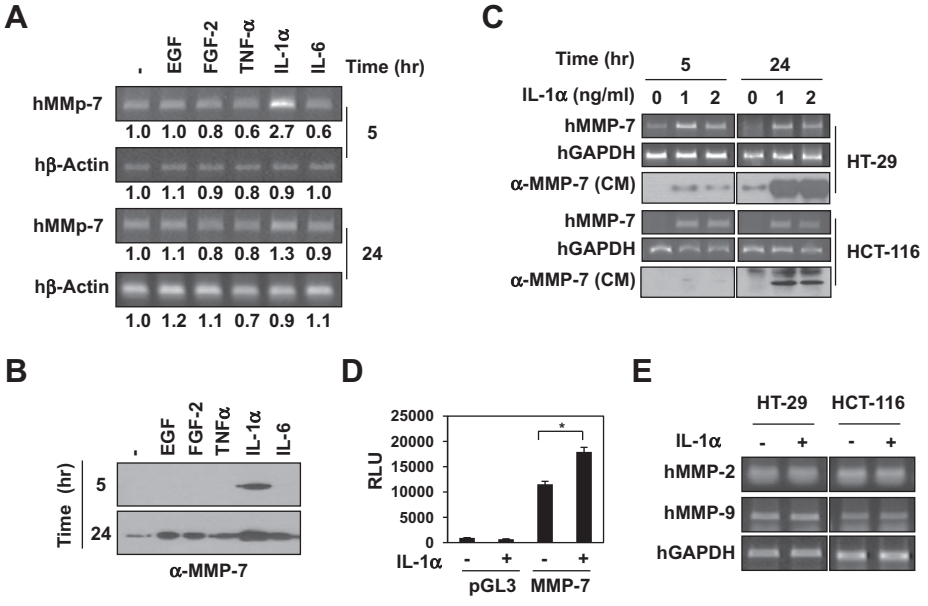


**Fig. 2.** IL-1α enhances expression of MMP-7 in colon cancer cells. (A) HT-29 cells were treated with the indicated factors for the indicated periods of time. Total RNA was extracted, and expression of MMP-7 was analyzed by RT-PCR. β-Actin was used as a loading control. Quantitative analysis of mRNA levels was performed using Image J program. (B) After starving in serum-free media overnight, HT-29 cells were treated with the indicated soluble factors. Conditioned media were collected, and proteins were concentrated by TCA precipitation and analyzed by immunoblotting using an anti-MMP-7 antibody. (C) After starving in serum-free media overnight, cells were treated with the indicated amount of IL-1α. *Top panel:* MMP-7 mRNA expression was analyzed by RT-PCR; GAPDH was used as a control. *Bottom panel:* Conditioned media were collected, and proteins were concentrated by TCA precipitation and analyzed by immunoblotting using an anti-MMP-7 antibody. (D) HCT-116 cell transfected with MMP-7 construct were treated with 1 ng/ml of IL-1α for 1 h. MMP-7 promoter activity was analyzed by luciferase assay. The mean of relative luciferase units (RLU) ± SEM are shown; $^{*}p < 0.05$. (E) Cells were treated with 1 ng/ml of IL-1α for 24 h. Total RNA was extracted, and expression of MMP-2 and -9 was analyzed by RT-PCR.

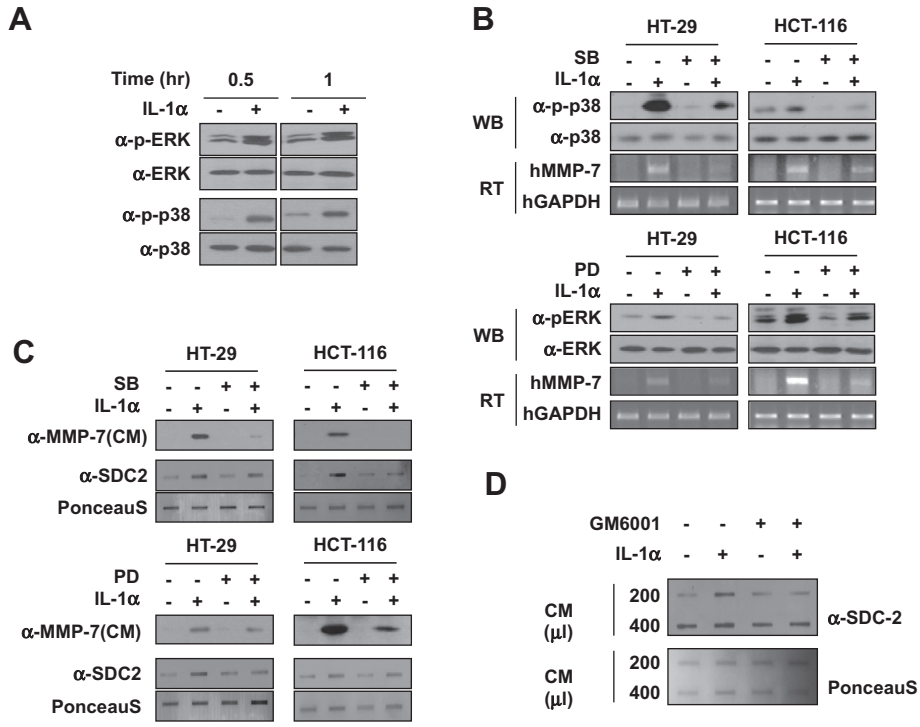


**Fig. 3.** IL-1α promotes expression of MMP-7 through the p38 MAP kinase signaling pathway. (A) After serum starving overnight, HT-29 cells were treated with 1 ng/ml of IL-1α for the indicated time. MAP kinase activation was then assessed in cell lysates using antibodies specific for phospho-p38 (p-p38) and -ERK (pERK). Antibodies against total p38 and ERK were used as controls for changes in protein expression. (B) HT-29 and HCT-116 cells were pre-treated with 1 ng/ml of IL-1α in the presence or absence of SB239063 (20 μM) or PD98059 (20 μM). *Top panel:* After 1-h incubation, cells were lysed and total cell lysates were immunoblotted with the indicated antibodies to determine the phosphorylation status of p38 and ERK. *Bottom panel:* After 5-h incubation, total RNA was extracted, and expression of MMP-7 was analyzed by RT-PCR. (C) HT-29 and HCT-116 cells were pre-treated with 1 ng/ml of IL-1α in the presence or absence of SB239063 or PD98059. After 24-h incubation, conditioned media were analyzed by immunoblotting using an anti-MMP-7 antibody (top panel) or slot blotting with an anti-syndecan-2 antibody (bottom panel). (D) HT-29 cells were treated with 1 ng/ml of IL-1α in the presence or absence of GM6001 (10 μM). After 24-h incubation, conditioned media were analyzed by slot blotting with an anti-syndecan-2 antibody. The volume of conditioned media (CM) used for slot blotting is indicated.

### *3.4. NF-κB is also involved in the regulation of IL-1α-induced syndecan-2 shedding in HT-29 colon cancer cells*

It is known that IL-1α regulates activation of NF-κB, a crucial mediator of the expression of inflammatory cytokines, including IL-6 and IL-8 [17]. Moreover, NF-κB regulates expression of MMP-7 in colon epithelial cells [23]. If MMP-7 plays a key role in the regulation of syndecan-2 shedding, the NF-κB signaling pathway might also be involved in IL-1α-induced syndecan-2 shedding in colon cancer cells. IL-1α enhanced nuclear localization of the NF-κB p65 subunit in HT-29 cells (Fig. 4A), suggesting that IL-1α induces activation of the NF-κB signaling pathway. In addition, Bay11-7085, an inhibitor of NF-κB activation, blocked IL-1α-induced nuclear localization of NF-κB-p65 in these cells (Fig. 4A). Interestingly, Bay11-7085 partially blocked MMP-7 mRNA expression and subsequently reduced the levels of secreted MMP-7 and shed syndecan-2 in conditioned media from HT-29 cells (Fig. 4B). In contrast, IL-1α had no effect on NF-κB-p65 activation in HCT-116 cells (Fig. 4A), suggesting that the IL-1α dependence of the NF-κB signaling pathway differs between HT-29 and HCT-116 cells. Taken together, these data suggest that NF-κB is involved in regulating IL-1α-induced syndecan-2 shedding in HT-29 colon cancer cells, and that MMP-7 expression is a key event in the IL-1α-dependent regulation of extracellular domain shedding of syndecan-2 in colon cancer cells.

## 4. Discussion

We previously reported that MMP-7 mediates extracellular shedding of syndecan-2 in colon cancer cells [10]. Although extracellular shedding of syndecan-2 could conceivably be regulated by various soluble factors, whether this was the case in colon cancer cells was not previously known. In the present study, we demonstrate that the proinflammatory cytokine, IL-1α, regulates extracellular shedding of syndecan-2. We showed that IL-1α promoted extracellular shedding of syndecan-2 (Fig. 1), expression of MMP-7 (Fig. 2) and phosphorylation of MAP kinase (Fig. 3), activation of NF-κB (Fig. 4), and established that activation of MAP kinase was critical for the expression of MMP-7 and extracellular shedding of syndecan-2 (Fig. 3). On the basis of these observations, we propose that IL-1α regulates extracellular domain shedding of syndecan-2 through regulation of MAP kinase-mediated MMP-7 expression in colon cancer cells.

A previous report showed that IL-1α secreted from colon cancer cells regulates tumor invasiveness and angiogenesis through induction of the expression of cancer-related genes [16]. Our data suggest that the gene encoding MMP-7 might be a critical target of IL-1α in colon cancer progression, and further that MMP-7 regulates colon cancer cell activity by regulating extracellular shedding of syndecan-2. In addition, our data showed that IL-1α-mediated extracellular shedding of syndecan-2 occurs in both early- and metastatic-stage colon cancer cells. Given that MMP-7 is an important regulator of colon cancer progression from the early stage to metastatic development and considered in light of the linkage between a proinflammatory cytokine (IL-1α) and two key players in colon cancer activities (MMP-7 and syndecan-2), our data have significant implications for the roles of IL-1α in colon cancer progression. Although further work is required to elucidate the roles of IL-1α and syndecan-2 shedding during colon cancer progression and establish the precise mechanism by which IL-1α mediates the

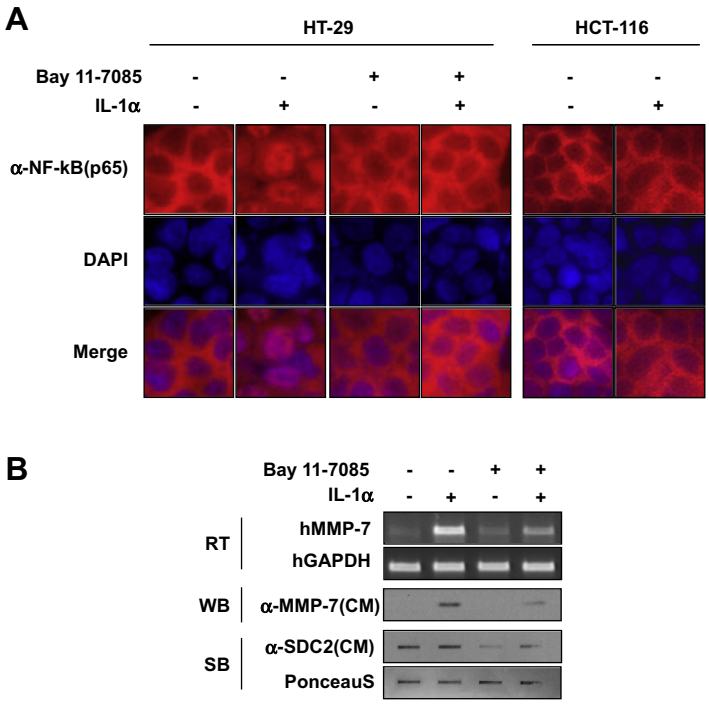


**Fig. 4.** NF-κB is also involved in regulating IL-1α-induced syndecan-2 shedding in HT29 colon cancer cells. (A) After starving in serum-free media overnight, HT-29 and HCT-116 cells were treated with 1 ng/ml of IL-1α in the absence or presence of Bay 11-7085 (20 μM). Cells were immunostained with an anti-NF-κB (p65) antibody. The results were visualized with a Texas Red-conjugated goat anti-rabbit antibody. DAPI was used to stain nuclei. (B) HT-29 cells were treated with the indicated amount of IL-1α in the absence or presence of Bay 11-7085 (20 μM). *Top panel:* MMP-7 mRNA expression was analyzed by RT-PCR; GAPDH was used as a control. *Bottom panel:* Conditioned media were analyzed by immunoblotting using an anti-MMP-7 antibody or slot blotting with an anti-syndecan-2 antibody.

expression of MMP-7 and extracellular shedding of syndecan-2, our present findings offer important new insights into the regulatory role of IL-1α in syndecan-2 shedding in colon cancer.

## Acknowledgments

This research was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korea Government (MEST) (No. 2012R1A5A1048236, 2013R1A2A2A01013565) and Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea. (HI12C0050).

## References

[1] Y.H. Wu, C.Y. Yang, W.L. Chien, K.I. Lin, M.Z. Lai, Removal of syndecan-1 promotes TRAIL-induced apoptosis in myeloma cells, J. Immunol. 188 (2012) 2914–2921.
[2] Y.B. Khotskaya, Y. Dai, J.P. Ritchie, V. MacLeod, Y. Yang, K. Zinn, R.D. Sanderson, Syndecan-1 is required for robust growth, vascularization, and metastasis of myeloma tumors in vivo, J. Biol. Chem. 284 (2009) 26085–26095.
[3] D.S. Choi, J.H. Kim, H.S. Ryu, H.C. Kim, J.H. Han, J.S. Lee, C.K. Min, Syndecan-1, a key regulator of cell viability in endometrial cancer, Int. J. Cancer 121 (2007) 741–750.
[4] J.R. Conejo, J. Kleeff, A. Koliopanos, K. Matsuda, Z.W. Zhu, H. Goecke, N. Bicheng, A. Zimmermann, M. Korc, H. Friess, M.W. Büchler, Syndecan-1 expression is up-regulated in pancreatic but not in other gastrointestinal cancers, Int. J. Cancer 88 (2000) 12–20.
[5] H. Park, I. Han, H.J. Kwon, E.S. Oh, Focal adhesion kinase regulates syndecan-2-mediated tumorigenic activity of HT1080 fibrosarcoma cells, Cancer Res. 65 (2005) 9899–9905.
[6] J.H. Lee, H. Park, H. Chung, S. Choi, Y. Kim, H. Yoo, T.Y. Kim, H.J. Hann, I. Seong, J. Kim, K.G. Kang, I.O. Han, E.S. Oh, Syndecan-2 regulates the migratory potential of melanoma cells, J. Biol. Chem. 284 (2009) 27167–27175.
[7] H.Y. Ryu, J. Lee, S. Yang, H. Park, S. Choi, K.C. Jung, S.T. Lee, J.K. Seong, I.O. Han, E.S. Oh, Syndecan-2 functions as a docking receptor for pro-matrix metalloproteinase-7 in human colon cancer cells, J. Biol. Chem. 284 (2009) 35692–35701.
[8] S.V. Subramanian, M.L. Fitzgerald, M. Bernfield, Regulated shedding of syndecan-1 and -4 ectodomains by thrombin and growth factor receptor activation, J. Biol. Chem. 272 (1997) 14713–14720.
[9] T. Manon-Jensen, Y. Itoh, J.R. Couchman, Proteoglycans in health and disease: the multiple roles of syndecan shedding, FEBS J. 277 (2010) 3876–3889.
[10] S. Choi, J.Y. Kim, J.H. Park, S.T. Lee, I.O. Han, E.S. Oh, The matrix metalloproteinase-7 regulates the extracellular shedding of syndecan-2 from colon cancer cells, Biochem. Biophys. Res. Commun. 417 (2012) 1260–1264.
[11] R.N. Apte, S. Dotan, M. Elkabets, M.R. White, E. Reich, Y. Carmi, X. Song, T. Dvozkin, Y. Krelin, E. Voronov, The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions, Cancer Metastasis Rev. 25 (2006) 387–408.
[12] C.A. Dinarello, Interleukin-1 and interleukin-1 antagonism, Blood 77 (1991) 1627–1652.
[13] L. Feldmeyer, S. Werner, L.E. French, H.D. Beer, Interleukin-1, inflammasomes and the skin, Eur. J. Cell Biol. 89 (2010) 638–644.
[14] J. Shao, H. Sheng, Prostaglandin E2 induces the expression of IL-1alpha in colon cancer cells, J. Immunol. 178 (2007) 4097–4103.
[15] A.M. Lewis, S. Varghese, H. Xu, H.R. Alexander, Interleukin-1 and cancer progression: the emerging role of interleukin-1 receptor antagonist as a novel therapeutic agent in cancer treatment, J. Transl. Med. 4 (2006) 48.
[16] Y. Matsuo, H. Sawai, J. Ma, D. Xu, N. Ochi, A. Yasuda, H. Takahashi, H. Funahashi, H. Takeyama, IL-1alpha secreted by colon cancer cells enhances angiogenesis: The relationship between IL-1alpha release and tumor cells' potential for liver metastasis, J. Surg. Oncol. 99 (2009) 361–367.
[17] M.U. Martin, H. Wesche, Summary and comparison of the signaling mechanisms of the Toll/interleukin-1 receptor family, Biochem. Biophys. Res. Commun. 1592 (2002) 265–280.
[18] Q. Li, P.W. Park, C.L. Wilson, W.C. Parks, Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury, Cell 111 (2002) 635–646.
[19] J. Xu, P.W. Park, F. Kheradmand, D.B. Corry, Endogenous attenuation of allergic lung inflammation by syndecan-1, J. Immunol. 174 (2005) 5758–5765.
[20] C. Seidel, O. Ringdén, M. Remberger, Increased levels of syndecan-1 in serum during acute graft-versus-host disease, Transplantation 76 (2003) 423–426.
[21] P.W. Park, G.B. Pier, M.T. Hinkes, M. Bernfield, Exploitation of syndecan-1 shedding by Pseudomonas aeruginosa enhances virulence, Nature 411 (2001) 98–102.
[22] B.L. Petrella, D.A. Armstrong, M.P. Vincenti, Interleukin-1 beta and transforming growth factor-beta 3 cooperate to activate matrix metalloproteinase expression and invasiveness in A549 lung adenocarcinoma cells, Cancer Lett. 325 (2012) 220–226.
[23] S.H. Lee, K.S. Song, D.H. Sohn, G.S. Seo, Polyozellin blocks tumor necrosis factor α-induced interleukin 8 and matrix metalloproteinase 7 production in the human intestinal epithelial cell line HT-29, Arch. Pharm. Res. 34 (2011) 91–97.