Biochemical and Biophysical Research Communications 452 (2014) 1084–1090

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

# Shedding of epithin/PRSS14 is induced by TGF-β and mediated by tumor necrosis factor-α converting enzyme

Hyo Seon Lee [a,1], Bo Mi Park [a,2], Youngkyung Cho [a], Sauryang Kim [b], Chungho Kim [c], Moon Gyo Kim [b], Dongeun Park [a,*]

[a] School of Biological Sciences, Seoul National University, Seoul 151-747, Republic of Korea
[b] Department of Biological Sciences, Inha University, Incheon 402-751, Republic of Korea
[c] School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea

ARTICLE INFO

*Article history:*
Received 10 September 2014
Available online 20 September 2014

*Keywords:*
Epithin
Ectodomain shedding
TGF-β
Tumor necrosis factor-α converting enzyme (TACE)

ABSTRACT

Epithin/PRSS14, a type II transmembrane serine protease, plays critical roles in cancer metastasis. Previously, we have reported that epithin/PRSS14 undergoes ectodomain shedding in response to phorbol myristate acetate (PMA) stimulation. In this study, we show that transforming growth factor-β (TGF-β) induces rapid epithin/PRSS14 shedding through receptor mediated pathway in 427.1.86 thymoma cells. Tumor necrosis factor-α converting enzyme (TACE) is responsible for this shedding. Amino acid sequence encompassing the putative shedding cleavage site of epithin/PRSS14 exhibit strong homology to the cleavage site of L-selectin, a known TACE substrate. TACE inhibitor, TAPI-0 and TACE siRNA greatly reduced TGF-β-induced epithin/PRSS14 shedding. TGF-β treatment induces translocation of intracellular pool of TACE to the membrane where epithin/PRSS14 resides. These findings suggest that TGF-β induces epithin/PRSS14 shedding by mediating translocation of epithin/PRSS14 sheddase, TACE, to the membrane.



## 1. Introduction

Ectodomain shedding of membrane protein is an important aspect of cell regulation, development, and cell–cell interaction [1]. It is shown that ectodomain shedding can be activated by various stimuli, ranging from UV irradiation to osmotic stress, inflammatory mediators, growth factors, and autocrine of cell intrinsic signaling events [2]. This shedding process regulates the fate and physical location of membrane-anchored growth factors [3], growth factor receptors [4], cytokine receptors, cell adhesion molecules [5], and proteins of unknown function such as the β-amyloid precursor protein (β-APP) [6].

Epithin/PRSS14 is broadly expressed in epithelial cells. Its expression is elevated in many carcinoma cells, and induced in macrophages by interferon-γ [7]. In physiological conditions, it plays essential roles in the maintenance of epithelial integrity, particularly epidermal terminal differentiation and barrier function [8]. When the gene is expressed in the skin by keratin 5 promoter, skin tumors appear spontaneously and tumorigenesis can be facilitated by chemical carcinogens or tumor promoters [9]. Earlier, we reported the critical roles of epithin/PRSS14 in angiogenesis [10], epithelial–mesenchymal transition (EMT) [11], and transendothelial migration *in vitro* and tumor metastasis of 4T1 breast cancer cells *in vivo* [7,12]. Soluble epithin/PRSS14 protein shed from the cancer cells shows the angiogenic activities [10]. These findings suggest that epithin/PRSS14 is involved in the various stages of cancer progression.

Epithin/PRSS14 undergoes post-translational processing during biogenesis and ectodomain shedding upon stimulation [10,13,14]. Epithin/PRSS14 is synthesized as a 110-kDa full length protein (Epi-L in Fig. 1A) followed by processing at Gly149 and expressed at membrane as a 92-kDa form (Epi-S in Fig. 1A) noncovalently connected to a short NH2-terminal fragment (18-kDa NTF in Fig. 1A). The epithin/PRSS14 can be further cleaved, presumably in autocatalytic fashion, at Arg614 between the 4th LDLRA domain and the serine protease domain, producing a two-chain but disulfide-linked activated form including 32-kDa protease domain (aEpi-S in Fig. 1A) [14]. The 88-kDa soluble form (Epi-S′ in

* Corresponding author at: School of Biological Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea. Fax: +82 2 886 0932.
*E-mail address:* depark@snu.ac.kr (D. Park).
[1] Present address: Department of Bio Therapeutics, Samsung Advanced Institute of Technology, Samsung Electronics Co., Ltd., Suwon 443-803, Republic of Korea.
[2] Present address: Department of Quality Assurance, Samsung biologics Co., Incheon 406-840, Republic of Korea.

http://dx.doi.org/10.1016/j.bbrc.2014.09.055
0006-291X/

Fig. 1A) of epithin/PRSS14 found in the culture medium may be generated by cleavage at Lys189/Arg204 and secreted into the culture medium [13]. It is known that the secreted form of epithin/PRSS14 can be converted to an active form that cleaves the ECM components including collagen, fibronectin and laminin that mediate cell attachment and migration [15].

TGF-β is a secreted homodimeric protein that regulates numerous cellular responses, such as proliferation, differentiation, migration, and apoptosis in addition to EMT in a context dependent fashion. TGF-β initiates its diverse cellular responses by binding to and activation of specific cell surface receptors that have intrinsic serine/threonine kinase activity [16,17]. Recently, involvement of TGF-β in the shedding of membrane proteins in cancer cells has been reported. In gastric cancer cells, TGF-β induces the shedding of membrane-anchored heparin-binding EGF-like growth factor and transactivates epidermal growth factor receptor (EGFR) [18]. In prostate cancer cells, TGF-β induces expression and shedding of the activated leukocyte cell adhesion molecule and enhances metastasis to bone [19]. In both case, the TGF-β-induced shedding is mediated through activation of TACE, also known as ADAM17.

TACE belongs to the ADAM (a disintegrin and metalloproteinase domain) family of proteins containing a zinc-dependent catalytic domain. It is implicated in ectodomain shedding of various growth factors, cytokines, receptors, and adhesion molecules [20,21]. There are many reports showing that TACE is involved in the PMA-induced shedding of various transmembrane proteins [22,23]. We reported that epithin/PRSS14 shedding is also induced by PMA treatment [13]. However, the upstream ligands and the sheddases responsible for epithin/PRSS14 shedding have not been reported.

In this study, we show that TGF-β induces rapid shedding of epithin/PRSS14 in 427.1.86 cells, and this shedding is mediated by TACE that translocates from cytosol to membrane upon TGF-β stimulation.

## 2. Materials and methods

### 2.1. Antibodies and reagents

Goat anti-TACE antibody was purchased from Santa Cruz Biotechnology. Mouse anti-β tubulin antibody was from Sigma. The mAb5 that recognizes the C-terminus of epithin/PRSS14 and anti-epithin polyclonal antibody were raised using GST-epithin/PRSS14 as antigen as previously described [14]. Various concentrations of $ZnCl_2$ and $MgCl_2$ were used to supply the cations. GM6001, SB431542, and TAPI-0 were purchased from Calbiochem. Recombinant human TGF-β was from R&D systems. Transfection was performed with METAFECTENE® SI reagent (Biontex). Control and TACE siRNA were purchased from Santa Cruz Biotechnology.

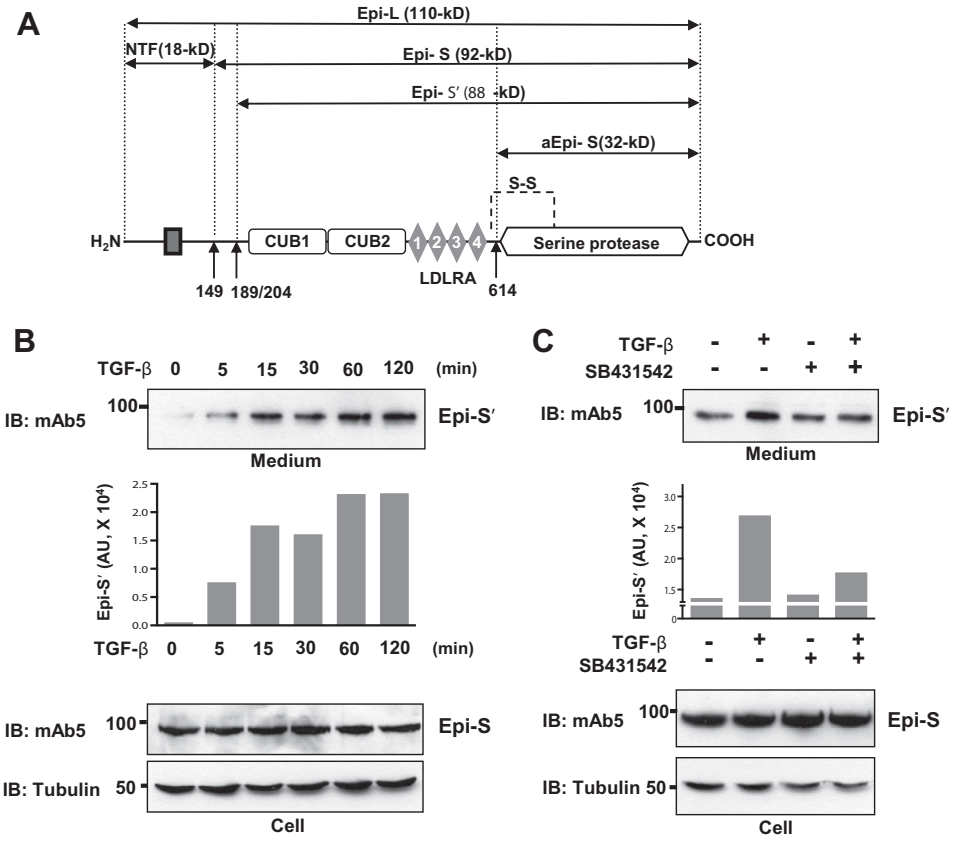


**Fig. 1.** TGF-β induces epithin/PRSS14 shedding. (A) Schematic diagram of epithin/PRSS14 domain structure. TM, transmembrane domain; CUB1, CUB2, complement subcomponent C1r/C1s domain; LDLRA, low density lipoprotein receptor class A repeats; serine protease domain. The activation cleavage site (Arg614) of epithin, and the connecting disulfide bonds (S–S) are shown. The Gly149 cleavage site for the generation of epithin/PRSS14 short form (Epi-S) and the putative cleavage sites for shedding (Lys189/Arg204) are also indicated. The sizes of various epithin/PRSS14 forms (Epi-L, Epi-S, Epi-S′, aEpi-S) found in the cell and medium are indicated above of the diagram. (B) Epithin/PRSS14 shedding is induced by TGF-β. 427.1.86 cells were treated with TGF-β for indicated time. Proteins in the media were precipitated with TCA and analyzed by Western blotting with mAb5 for epithin/PRSS14 (upper panel). Quantitation of Epi-S′ bands are shown in the graph below. The amounts of Epi-S and β-tubulin in the cell lysates are also shown (lower panel). (C) Inhibition of TGF-β receptor reduces epithin/PRSS14 shedding. 427.1.86 cells were pretreated with 10 μM SB431542, a TGF-β type I receptor kinase inhibitor, for 30 min and treated with TGF-β for 2 h. Epithin/PRSS14 proteins in the media and the cell lysates were analyzed and quantitated as described in (B).

### 2.2. Cell culture and transfection

427.1.86 cells [24] were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL) with 10% Fetal Bovine Serum (FBS, Gibco-BRL) and antibiotics (Gibco-BRL) at 37 °C in 10% $CO_2$ incubator. Transient transfection of the cell suspension was performed using METAFECTENE® SI reagent (Biontex) according to manufacturer's protocol. Mixture of the METAFECTENE® SI and TACE siRNA in 1X SI buffer was incubated for 15 min at room temperature. Then the cell suspension was added to the well containing the lipoplex and incubated without further mixing. Epithin/PRSS14 shedding and the effect of inhibitors were examined at 24 h after the addition of the lipoplex.

### 2.3. Western blot analysis

427.1.86 cell lysates and the 10% trichloroacetic acid (TCA)-precipitates from conditioned medium were separated on 9% acrylamide gel under reducing condition. After SDS–polyacrylamide gel electrophoresis, proteins were transferred onto nitrocellulose (NC) membrane (Whatman). The membrane was blocked with 5% skim milk and reacted with mAb5 (for Epi-S′ and aEpi-S), anti-TACE antibody, anti-β tubulin, or anti-GAPDH antibody for 1 h. After washing 3 times with PBS containing 0.1% Triton X-100 (PBS-T), the membrane was incubated with horseradish peroxidase-conjugated secondary antibody and washed 5 times with PBS-T, then visualized using Enhanced Chemiluminescence reagents (Supersignal®West Femto, Thermo).

### 2.4. Immunocytochemistry

427.1.86 cells were grown on 0.1% gelatin-coated cover slip. After 1 day (cell confluency at 80%), cells were incubated with serum-free media for 4 h and then treated with 5 ng/ml TGF-β for indicated time, and fixed with 3.7% paraformaldehyde in PBS. Then cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min, washed twice, and incubated with blocking solution (10% donkey serum and 0.1% Triton X-100 in PBS) for 30 min. After washing twice with PBS-T, samples were treated with anti-epithin polyclonal antibody or anti-TACE antibody (Santa Cruz Biotechnology, #6416) in blocking solution. After 1 h, cells were washed twice with PBS-T and then incubated with FITC conjugated secondary antibodies (Jackson ImmunoResearch) for 50 min. For actin staining, rhodamine-phalloidin (Molecular Probe, R415) was used. Cells were observed under the fluorescence microscope (Axioplan200 M, Carl Zeiss) equipped with a 100× objective lens. Images were processed in photoshop CS6 (Adobe).

### 2.5. Sequence alignment and protein modeling

Amino acid sequences of epithin/PRSS14 and L-selectin from three different species (human, mouse, and rat) were obtained from NCBI. Accession numbers for the amino acid sequences used are human matriptase/PRSS14 (hST14), Q9Y5Y6, mouse epithin/PRSS14 (mST14), P56677, rat epithin/PRSS14 (rST14), AAH97271, human selectin (hSEL), P14151, mouse selectin (mSEL), P18337, rat selectin (rSEL), P30836. The sequences of the extracted amino acid were clustered by taking 13-mer and 21-mer encompassing the putative cleavage sites using ClustalW package in the MacVector program. The tertiary structure of matriptase/PRSS14 (residues 150-855) was predicted by using I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) and analyzed with Chimera program (https://www.cgl.ucsf.edu/chimera/).

## 3. Results

### 3.1. TGF-β induces epithin/PRSS14 shedding

While we were studying the effects of TGF-β on EMT process in 427.1.86 cells [11], we noticed the rapid shedding of epithin/PRSS14 into media (Fig. 1B). Upon TGF-β treatment, the 88-kDa shed epithin/PRSS14 (Epi-S′) in the medium was increased. Shedding of epithin/PRSS14 was detected as early as at 5 min after TGF-β treatment and reached to maxim within 1 h, suggesting that TGF-β may directly activate epithin/PRSS14 shedding. This rapid effect of TGF-β on epithin/PRSS14 shedding is a contrast to the delayed effects of TGF-β on epithin/PRSS14 upregulation that was observed after 24 h of TGF-β treatment [11]. TGF-β-induced epithin/PRSS14 shedding was drastically reduced by pre-treatment of the TGF-β type I receptor kinase inhibitor SB431542, indicating that the effect of TGF-β on epithin/PRSS14 shedding depends on receptor-mediated signaling processes (Fig. 1C).

### 3.2. A cation-dependent metalloproteinase, TACE mediates epithin/PRSS14 shedding

To investigate whether the epithin/PRSS14 shedding is dependent on divalent cations, the effects of various concentrations of $Zn^{2+}$ and $Mg^{2+}$ on epithin/PRSS14 shedding were examined. As shown in Fig. 2A, 0.1 mM $Zn^{2+}$ and 10 mM $Mg^{2+}$ dramatically increased the amount of shed epithin/PRSS14 (Epi-S′) in the medium. These results suggest that metalloproteinase may be involved in the shedding process. To verify the involvement of metalloproteinase, a general metalloproteinase inhibitor, GM6001 was included in the experiments. GM6001 significantly reduced the metal ion-induced epithin/PRSS14 shedding. In addition to the increase in epithin/PRSS14 shedding, divalent cations also induced activation of epithin/PRSS14 as revealed by the appearance of active form of aEpi-S (32-kDa) in the medium. These results suggest that $Zn^{2+}$-induced shedding also facilitates the activation of epithin/PRSS14.

Since a metalloproteinase, TACE is implicated in ectodomain shedding of many membrane proteins [25,26], we tested whether the $Zn^{2+}$-dependent epithin/PRSS14 shedding is blocked by TACE inhibitor, TAPI-0. Not surprisingly, 10 μM TAPI-0 inhibited $Zn^{2+}$-dependent epithin/PRSS14 shedding by 80% indicating that TACE is a major sheddase for $Zn^{2+}$-dependent epithin/PRSS14 shedding (Fig. 2B). In addition, TAPI-0 treatment also reduced the amount of active aEpi-S to the similar extent as the shed Epi-S′ was reduced. This result suggests that shedding and activation of epithin/PRSS14 are closely coupled.

When we aligned the target sequences of the various known substrates of TACE with the putative cleavage sequences of epithin/PRSS14 (the sequences encompassing Lys189 and Arg204), we found that amino acid conservation among the cleavage sites in total is very poor (data not shown). This result is not unexpected since TACE is known to have very relaxed sequence specificity for substrate cleavage sites. However, there is a significant conservation between the sequences encompassing the putative cleavage site (Lys189) of epithin/PRSS14 and the cleavage site of L-selectin, a well known TACE substrate. Interestingly, three amino acid residues (KSF) near Lys189 cleavage site of epithin/PRSS14 is identical with those of L-selectin (Fig. 2C, and [26]). Conservation between Lys189 site of epithin/PRSS14 and Lys321 site of L-selectin extends further as shown in Fig. 2C. In a model structure, Lys189 cleavage site of epithin/PRSS14 is well exposed and the conserved sequences are located in the position that can be recognized easily by the sheddase (Fig. 2D). The sequence similarity surrounding the cleavage site of L-selectin and the putative cleavage site of epithin/

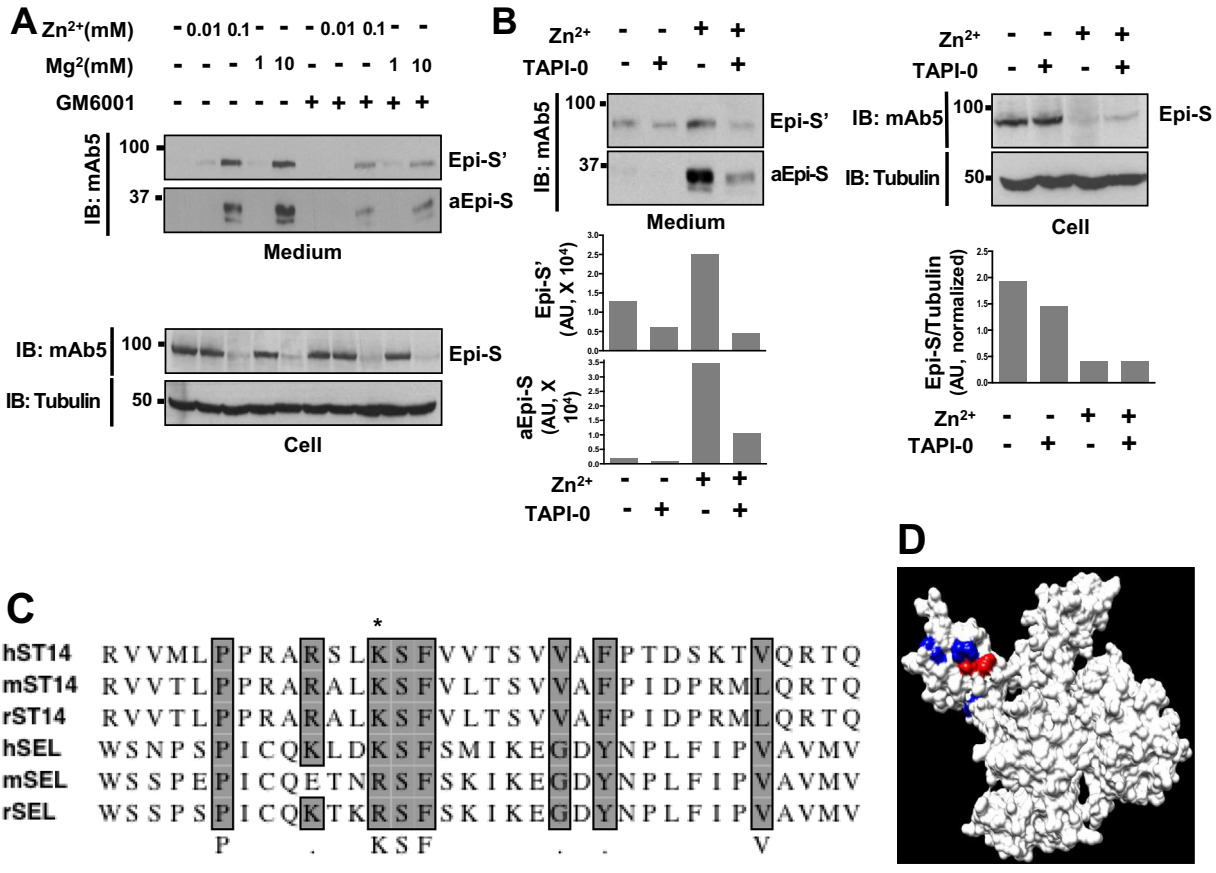


**Fig. 2.** TACE mediates epithin/PRSS14 shedding induced by metal ions. (A) Divalent cation-sensitive metalloproteinase induces epithin/PRSS14 shedding. 427.1.86 cells were pretreated without or with 50 μM GM6001 for 2 h before divalent cations treatment. Proteins in the media were precipitated with TCA and analyzed by Western blotting with mAb5. Epithin/PRSS14 proteins in the cell lysates were also analyzed by Western blotting with mAb5. Epi-S′ and aEpi-S in the media are shown in upper panels. The amounts of Epi-S and β-tubulin in the cell lysates are shown in lower panels. (B) TACE inhibitor, TAPI-0 blocks $Zn^{2+}$-induced epithin/PRSS14 shedding. 427.1.86 cells were pretreated with 10 μM TAPI-0 before 0.1 m M $Zn^{2+}$ treatment. Proteins in the media were precipitated with TCA and analyzed by Western blotting with mAb5. Quantitations of the epithin/PRSS14 proteins in the media and the cell lysates are shown in the graphs below. (C) Comparison of the TACE cleavage sites of epithin/PRSS14 and L-selectin. The protein sequences encompassing the putative cleavage site (Lys189) of epithin/PRSS14 and the known TACE cleavage site (Lys321) of L-selectin are compared. The protein sequences from three different species (human, mouse, rat) are aligned. The "*" above the sequence alignments indicates the TACE cleavage sites. Gray box: identities and similarities (threshold: 51%). (D) Positions of the putative cleavage site (Lys189) in a model from the tertiary structure prediction of matriptase, the human orthologue of epithin/PRSS14. Red: Lys189–Ser190, blue: conserved residues.

PRSS14 provides another circumstantial evidence that TACE is a sheddase for epithin/PRSS14.

### 3.3. TACE mediates TGF-β-induced epithin/PRSS14 shedding

Next, we tested whether the TGF-β-induced epithin/PRSS14 shedding is sensitive to the TACE inhibitor, TAPI-0. As shown in Fig 3A, TGF-β-induced epithin/PRSS14 shedding in 427.1.86 cells was markedly inhibited by TAPI-0. This result suggests that TACE plays a major role in TGF-β-induced epithin/PRSS14 shedding. To further verify the involvement of TACE in the process, TGF-β-induced epithin/PRSS14 shedding was examined in the 427.1.86 cells where TACE expression was down-regulated by siRNA of TACE. As shown in Fig. 3B, knock down of TACE significantly inhibits TGF-β-induced epithin/PRSS14 shedding by 75% (1.2–0.3). Therefore it was concluded that TACE is the major sheddase for TGF-β-induced epithin/PRSS14 shedding.

### 3.4. TGF-β induces translocation of TACE from cytosol to membrane

To act as an epithin/PRSS14 sheddase, TACE should colocalized with epithin/PRSS14 in the plasma membrane in a TGF-β-dependent manner. Therefore, we investigated the changes in cellular localization of TACE upon TGF-β treatment. In untreated cells, TACE is located mainly in the vesicles as a punctate pattern in the cytosol (Fig. 4A, TGF-β 0 min). When treated with TGF-β, the gradual translocation of TACE from cytosol to membrane was clearly visible (Fig. 4A, TGF-β 0-120 min). In earlier time points, up to 30 min of the treatment, TACE was only partially located in the membrane. However, 2 h after the treatment, TACE was found on the cell membrane in nearly all the cells. In contrast, the changes in membrane localization of epithin/PRSS14 were less evident since the majority of epithin/PRSS14 is already localized in the membrane prior to TGF-β treatment in most cells (Fig. 4B). However, membrane localization of epithin/PRSS14 is somewhat enhanced upon TGF-β treatment. TGF-β also induced actin rearrangement as seen in Fig. 4. Within 2 h of TGF-β treatment, cortical actin becomes dominant, suggesting that actin rearrangement may be involved in the translocation of TACE and epithin/PRSS14. These results suggest that TGF-β induces TACE translocation from cytosol to membrane where its substrate, epithin/PRSS14 resides.

## 4. Discussion

Soluble form of epithin/PRSS14 protein is important in the regulation of cancer progression and angiogenesis. It induces endothelial cell migration, and vessel morphogenesis [10]. The pleiotropic

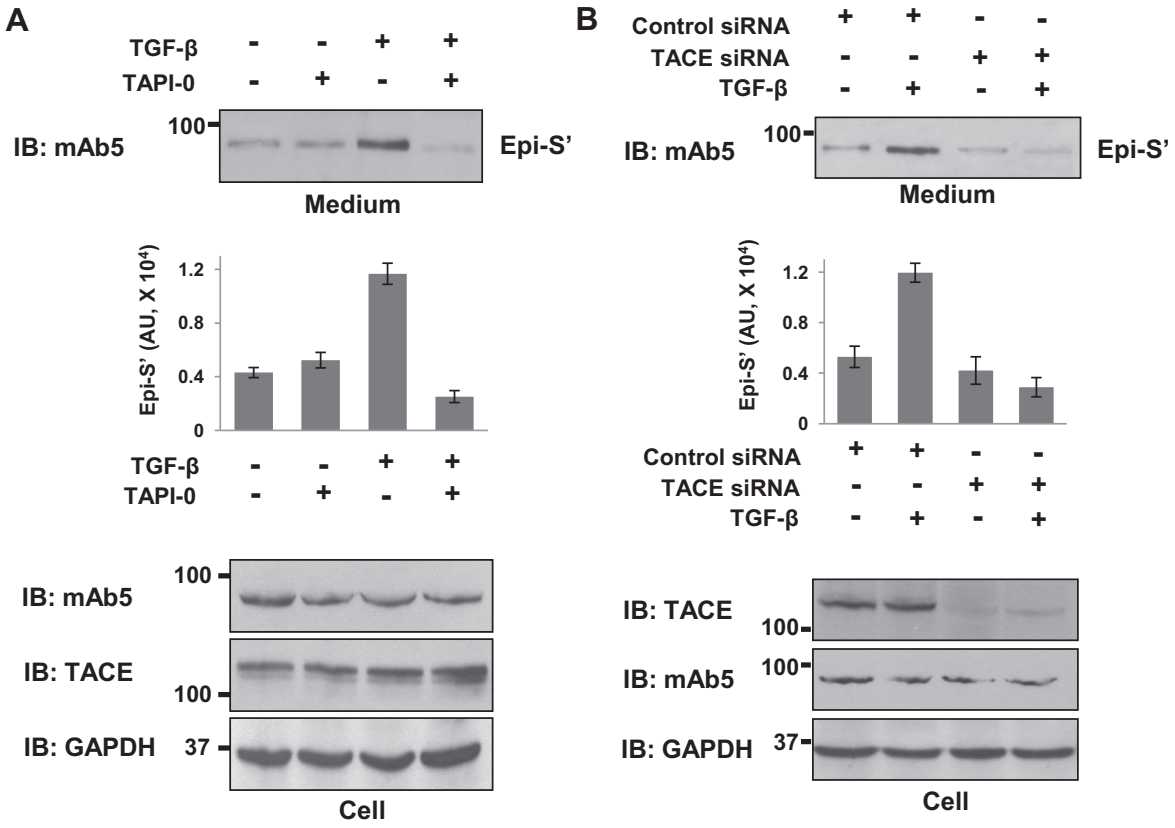


**Fig. 3.** TACE mediates TGF-β-induced epithin/PRSS14 shedding. (A) TACE inhibitor (TAPI-0) blocks TGF-β-induced epithin/PRSS14 shedding. 427.1.86 cells were pretreated without or with 10 μM TAPI-0 before incubation with 5 ng/ml TGF-β for 2 h. Proteins in the media were precipitated with TCA and analyzed by Western blotting with mAb5. Bar graph shows quantitation of Epi-S′ in the media from data obtained in three independent experiments. Proteins in the cell lysates were also analyzed by Western blotting with mAb5, anti-TACE, and GAPDH antibodies. (B) TACE knock down inhibits TGF-β-induced epithin/PRSS14 shedding. 427.1.86 cells were transfected with a siRNA of TACE or control siRNA as indicated. Proteins in the media were precipitated with TCA and analyzed by Western blotting with mAb5. Bar graph shows quantitation of Epi-S′ in the media from data obtained in three independent experiments. The amounts of TACE, epithin/PRSS14 and GAPDH in the cell lysates are also shown.

cytokine, TGF-β that plays important roles in various stages of tumorigenesis induces rapid shedding of epithin/PRSS14 (Fig. 1). Using specific inhibitor for TGF-β receptor, we provided evidence that the receptor-mediated signaling is involved in the activation of sheddase (Fig. 2). The shedding was observed as early as 5 min upon TGF-β treatment. This immediate effect implies that TGF-β directly activates signaling cascades through the receptor leading to the activation of the pertinent sheddase.

Previously, sphingosine 1-phosphate (S1P), present in serum-derived lipoproteins, was shown to rapidly activate and induce shedding of epithin/PRSS14 in human cancer cells [27,28]. However, this action of S1P does not seem to be mediated by the known S1P receptors, but by unidentified pathway. Steroid sex hormones such as 5α-dihydrotestosterone (DHT) were also shown to induce activation and shedding of epithin/PRSS14 but only after 6 h of the treatment. Thus, the action of DHT seems to be indirect and requires transcription of the hormone-targeted genes [29]. Therefore, as far as we know, TGF-β-induced epithin/PRSS14 shedding described in this study is the first report for the identification of an endogenous ligand that induces epithin/PRSS14 shedding through acting on a known receptor.

TGF-β is known to promote tumor cell invasion and metastasis through the induction of EMT [16]. In our earlier studies, the inhibition of TGF-β-induced EMT in 427.1.86 cells by epithin/PRSS14 knock down indicated that epithin/PRSS14 is an essential mediator of TGF-β-induced EMT [11]. We also reported that the shed epithin/PRSS14 collected from 427.1.86 cell conditioned medium enhances cell motility and contains active angiogenic potential [10]. Together with these results, we propose that TGF-β-induced epithin/PRSS14 shedding plays important roles in tumor metastasis and angiogenesis.

Matrix metalloproteinases (MMPs) participate in cell migration and remodeling process by affecting the extracellular matrix. All MMPs including TACE contain $Zn^{2+}$ in catalytic domain that is essential for substrate binding and cleavage [30]. Although the results shown in this study strongly suggest that divalent cations induce epithin/PRSS14 shedding through activation of TACE, we cannot exclude involvement of other MMPs in the shedding process. However, the blocking of epithin/PRSS14 shedding close to the control level by TACE knock down (Fig. 3B) indicates that TACE is the major sheddase for the divalent cation- and TGF-β-induced epithin/PRSS14 shedding.

Although TGF-β induces TACE-mediated epithin/PRSS14 shedding, the signaling mechanism how TGF-β induces epithin/PRSS14 shedding is still unclear. Previously, we have reported that the PMA-induced epithin/PRSS14 shedding requires rearrangement of actin cytoskeleton from stress fiber to cortical actin cytoskeleton [13,14]. PMA induces translocation of epithin/PRSS14 from cytosol to the membrane surface in an actin cytoskeleton-dependent manner to increase its local concentration that is required for the increase of epithin/PRSS14 shedding. On the basis of these findings and result obtained from immunocytochemistry shown in Fig. 4, we propose that TGF-β-dependent epithin/PRSS14 shedding also requires actin rearrangement in 427.1.86 cells.

TACE resides in the vesicles in unstimulated cells (Fig. 4 and [31,32]). It is known that treatment with TGF-β induces phosphor-

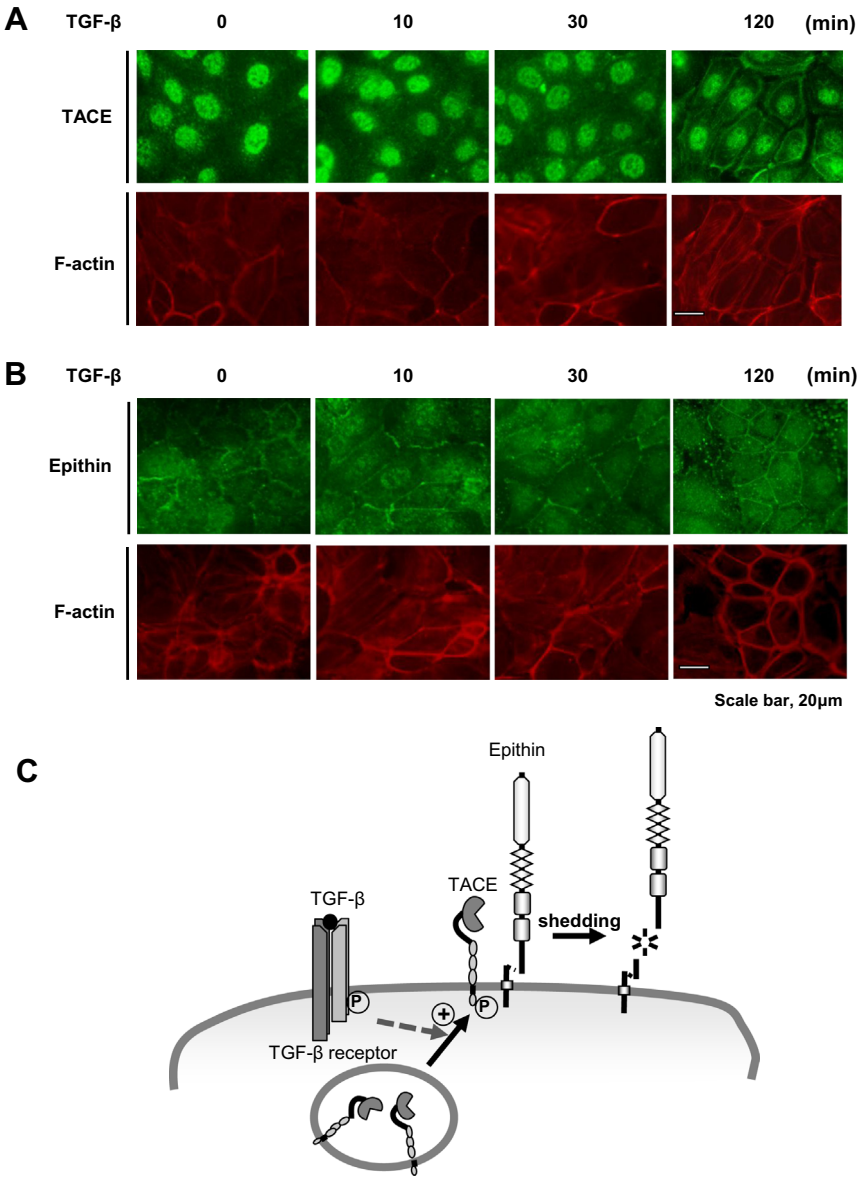


**Fig. 4.** TGF-β induces translocation of TACE from cytosol to membranes. (A,B) Cellular localizations of TACE and epithin/PRSS14. 427.1.86 cells were treated with TGF-β for indicated time. Then the cells were fixed and subjected to immunofluorescence staining. The cellular localizations of TACE and epithin/PRSS14 were analyzed by fluorescence microscopy after staining with anti-TACE antibody, and anti-epithin antibody, respectively. F-actins were stained with rhodamine-conjugated phalloidin. Scale bars, 20 μm. (C) Model for TGF-β induced epithin/PRSS14 shedding. TGF-β induces translocation of TACE from cytosol to cell membrane where epithin/PRSS14 resides and enhances its shedding.

ylation of TACE and its translocation to the cell surface [31,32]. In addition, Liu et al. [2] reported TGF-β upregulates EGFR ligand shedding through a mechanism involving the phosphorylation of TACE. We also found the phosphorylation of TACE (data not known) and translocation of TACE from cytosol to the cell surface upon TGF-β treatment. A model for the TGF-β-induced epithin/PRSS14 shedding that is mediated by TGF-β-dependent translocation of TACE to the cell surface where epithin/PRSS14 resides is proposed as shown in Fig. 4C.

## Acknowledgments

This work was supported by the Grant from the National Research Foundation of Korea (NRF-2011-0009555) to D.P. and by Inha University Research Grant and the Korea Healthcare Technology R&D Project (A111927), Ministry of Health & Welfare to M.G.K. H.S.L., B.M.P., and Y.C. were the recipients of the BK21 fellowship. None of the authors of this work has financial interest related to this work.

## References

[1] K. List, T.H. Bugge, R. Szabo, Matriptase: potent proteolysis on the cell surface, Mol. Med. 12 (2006) 1–7.
[2] C. Liu, P. Xu, S. Lamouille, J. Xu, R. Derynck, TACE-mediated ectodomain shedding of the type I TGF-beta receptor downregulates TGF-beta signaling, Mol. Cell 35 (2009) 26–36.
[3] J. Massague, A. Pandiella, Membrane-anchored growth factors, Annu. Rev. Biochem. 62 (1993) 515–541.
[4] S. Rose-John, P.C. Heinrich, Soluble receptors for cytokines and growth factors: generation and biological function, Biochem. J. 300 (Pt. 2) (1994) 281–290.
[5] M.R. Ehlers, J.F. Riordan, Membrane proteins with soluble counterparts: role of proteolysis in the release of transmembrane proteins, Biochemistry 30 (1991) 10065–10074.
[6] C. Haass, D.J. Selkoe, Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide, Cell 75 (1993) 1039–1042.
[7] D. Lee, H.S. Lee, S.J. Yang, H. Jeong, D.Y. Kim, S.D. Lee, J.W. Oh, D. Park, M.G. Kim, PRSS14/epithin is induced in macrophages by the IFN-gamma/JAK/STAT

pathway and mediates transendothelial migration, Biochem. Biophys. Res. Commun. 405 (2011) 644–650.
[8] K. List, C.C. Haudenschild, R. Szabo, W. Chen, S.M. Wahl, W. Swaim, L.H. Engelholm, N. Behrendt, T.H. Bugge, Matriptase/MT-SP1 is required for postnatal survival, epidermal barrier function, hair follicle development, and thymic homeostasis, Oncogene 21 (2002) 3765–3779.
[9] K. List, R. Szabo, A. Molinolo, V. Sriuranpong, V. Redeye, T. Murdock, B. Burke, B.S. Nielsen, J.S. Gutkind, T.H. Bugge, Deregulated matriptase causes ras-independent multistage carcinogenesis and promotes ras-mediated malignant transformation, Genes Dev. 19 (2005) 1934–1950.
[10] S.B. Kim, D. Lee, J.W. Jeong, C. Kim, D. Park, M.G. Kim, Soluble epithin/PRSS14 secreted from cancer cells contains active angiogenic potential, Mol. Cells 29 (2010) 617–623.
[11] H.S. Lee, C. Kim, S.B. Kim, M.G. Kim, D. Park, Epithin, a target of transforming growth factor-beta signaling, mediates epithelial–mesenchymal transition, Biochem. Biophys. Res. Commun. 395 (2010) 553–559.
[12] C. Kim, H.S. Lee, D. Lee, S.D. Lee, E.G. Cho, S.J. Yang, S.B. Kim, D. Park, M.G. Kim, Epithin/PRSS14 proteolytically regulates angiopoietin receptor Tie2 during transendothelial migration, Blood 117 (2011) 1415–1424.
[13] C. Kim, Y. Cho, C.H. Kang, M.G. Kim, H. Lee, E.G. Cho, D. Park, Filamin is essential for shedding of the transmembrane serine protease, epithin, EMBO Rep. 6 (2005) 1045–1051.
[14] E.G. Cho, M.G. Kim, C. Kim, S.R. Kim, I.S. Seong, C. Chung, R.H. Schwartz, D. Park, N-terminal processing is essential for release of epithin, a mouse type II membrane serine protease, J. Biol. Chem. 276 (2001) 44581–44589.
[15] S. Satomi, Y. Yamasaki, S. Tsuzuki, Y. Hitomi, T. Iwanaga, T. Fushiki, A role for membrane-type serine protease (MT-SP1) in intestinal epithelial turnover, Biochem. Biophys. Res. Commun. 287 (2001) 995–1002.
[16] P. ten Dijke, C.S. Hill, New insights into TGF-beta-Smad signalling, Trends Biochem. Sci. 29 (2004) 265–273.
[17] H. Ikushima, K. Miyazono, TGFbeta signalling: a complex web in cancer progression, Nat. Rev. Cancer 10 (2010) 415–424.
[18] M. Ebi, H. Kataoka, T. Shimura, E. Kubota, Y. Hirata, T. Mizushima, T. Mizoshita, M. Tanaka, M. Mabuchi, H. Tsukamoto, S. Tanida, T. Kamiya, S. Higashiyama, T. Joh, TGFbeta induces proHB-EGF shedding and EGFR transactivation through ADAM activation in gastric cancer cells, Biochem. Biophys. Res. Commun. 402 (2010) 449–454.
[19] A.G. Hansen, S.A. Arnold, M. Jiang, T.D. Palmer, T. Ketova, A. Merkel, M. Pickup, S. Samaras, Y. Shyr, H.L. Moses, S.W. Hayward, J.A. Sterling, A. Zijlstra, ALCAM/CD166 is a TGF-beta-responsive marker and functional regulator of prostate cancer metastasis to bone, Cancer Res. 74 (2014) 1404–1415.
[20] J. Scheller, A. Chalaris, C. Garbers, S. Rose-John, ADAM17: a molecular switch to control inflammation and tissue regeneration, Trends Immunol. 32 (2011) 380–387.
[21] R. Menghini, L. Fiorentino, V. Casagrande, R. Lauro, M. Federici, The role of ADAM17 in metabolic inflammation, Atherosclerosis 228 (2013) 12–17.
[22] R. Mezyk, M. Bzowska, J. Bereta, Structure and functions of tumor necrosis factor-alpha converting enzyme, Acta Biochim. Pol. 50 (2003) 625–645.
[23] A.P. Huovila, A.J. Turner, M. Pelto-Huikko, I. Karkkainen, R.M. Ortiz, Shedding light on ADAM metalloproteinases, Trends Biochem. Sci. 30 (2005) 413–422.
[24] S.J. Faas, J.L. Rothstein, B.L. Kreider, G. Rovera, B.B. Knowles, Phenotypically diverse mouse thymic stromal cell lines which induce proliferation and differentiation of hematopoietic cells, Eur. J. Immunol. 23 (1993) 1201–1214.
[25] N.L. Tsakadze, S.D. Sithu, U. Sen, W.R. English, G. Murphy, S.E. D'Souza, Tumor necrosis factor-alpha-converting enzyme (TACE/ADAM-17) mediates the ectodomain cleavage of intercellular adhesion molecule-1 (ICAM-1), J. Biol. Chem. 281 (2006) 3157–3164.
[26] D.M. Smalley, K. Ley, L-selectin: mechanisms and physiological significance of ectodomain cleavage, J. Cell Mol. Med. 9 (2005) 255–266.
[27] C. Benaud, R.B. Dickson, C.Y. Lin, Regulation of the activity of matriptase on epithelial cell surfaces by a blood-derived factor, Eur. J. Biochem. 268 (2001) 1439–1447.
[28] C. Benaud, M. Oberst, J.P. Hobson, S. Spiegel, R.B. Dickson, C.Y. Lin, Sphingosine 1-phosphate, present in serum-derived lipoproteins, activates matriptase, J. Biol. Chem. 277 (2002) 10539–10546.
[29] K. Kiyomiya, M.S. Lee, I.C. Tseng, H. Zuo, R.J. Barndt, M.D. Johnson, R.B. Dickson, C.Y. Lin, Matriptase activation and shedding with HAI-1 is induced by steroid sex hormones in human prostate cancer cells, but not in breast cancer cells, Am. J. Physiol. Cell Physiol. 291 (2006) C40–49.
[30] F. Fouchier, C. Penel, M. Pierre Montero, B. Bremond, S. Champion, Integrin alphavbeta6 mediates HT29-D4 cell adhesion to MMP-processed fibrinogen in the presence of Mn2+, Eur. J. Cell Biol. 86 (2007) 143–160.
[31] S.E. Wang, B. Xiang, M. Guix, M.G. Olivares, J. Parker, C.H. Chung, A. Pandiella, C.L. Arteaga, Transforming growth factor beta engages TACE and ErbB3 to activate phosphatidylinositol-3 kinase/Akt in ErbB2-overexpressing breast cancer and desensitizes cells to trastuzumab, Mol. Cell. Biol. 28 (2008) 5605–5620.
[32] S.E. Wang, B. Xiang, R. Zent, V. Quaranta, A. Pozzi, C.L. Arteaga, Transforming growth factor beta induces clustering of HER2 and integrins by activating Src-focal adhesion kinase and receptor association to the cytoskeleton, Cancer Res. 69 (2009) 475–482.