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

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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

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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.

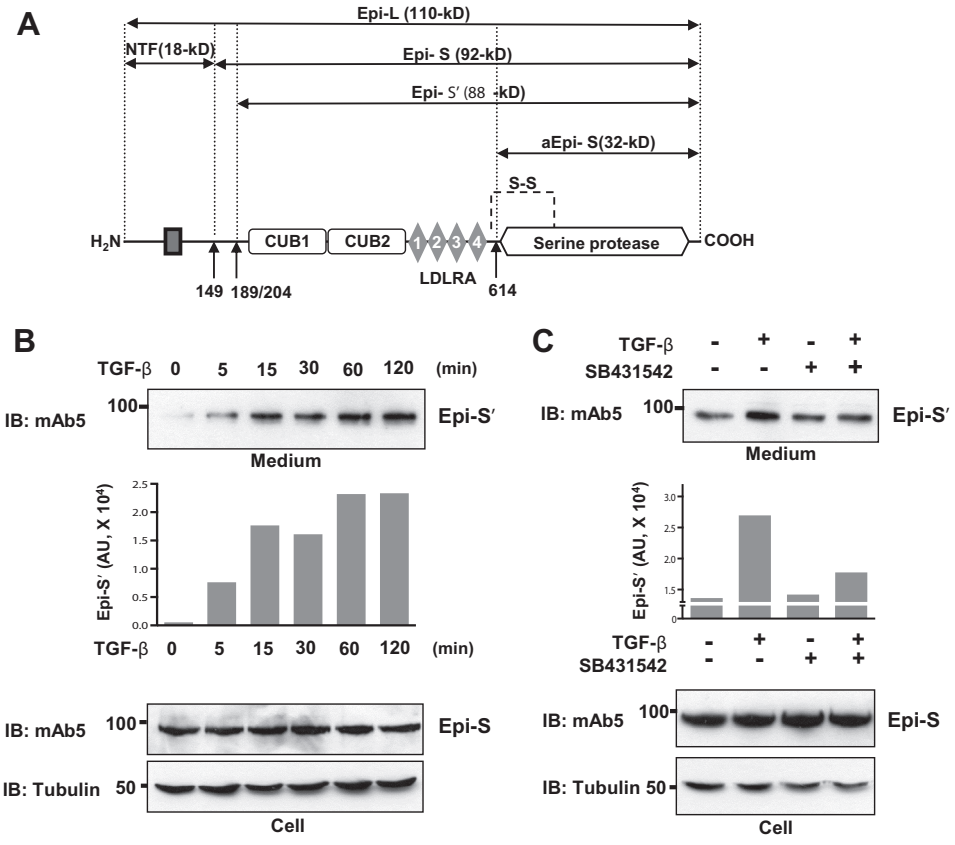

Fig. 1. TGF-β induces epithin/PRSS14 shedding. (A) Schematic diagram of epithin/PRSS14 domain structure. TM, transmembrane domain; CUB1, CUB2, complement subcomponent C1r/C1s domain; LDLRA, low density lipoprotein receptor class A repeats; serine protease domain. The activation cleavage site (Arg614) 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).