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Epithin, a target of transforming growth factor-β signaling, mediates epithelial–mesenchymal transition

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ABSTRACT

The epithelial-derived type II transmembrane serine protease epithin has been shown to be upregulated in a variety of cancer cell lines and tumor tissues, and its upregulation correlates well with tumor progression in many cases. However, little is known regarding the regulation of its expression and the mechanism of its roles in tumor progression. Here, we show that transforming growth factor-β (TGF-β), a potent inducer of epithelial–mesenchymal transition (EMT) in tumor progression, upregulates epithin, and that epithin plays a critical role in TGF-β-induced EMT. Forced overexpression of epithin induced EMT to exhibit characteristic morphological changes, alternations in EMT-related proteins and enhanced cell motility. Conversely, shRNA-mediated knockdown of endogenous epithin inhibited TGF-β-induced expression of mesenchymal markers and morphological changes. Furthermore, TGF-β-induced cell migration and invasion were significantly impaired by epithin knockdown. In addition, we demonstrate that TGF-β upregulates epithin transcriptionally via the Smad2/Smad4-mediated pathway. These results suggest that epithin is a key mediator of TGF-β-induced EMT in tumor progression.

1. Introduction

Epithelial–mesenchymal transition (EMT) is a differentiation switch from polarized epithelial cells to motile mesenchymal cells, and is a central process during embryonic development, fibrosis, and cancer progression [1]. In tumor progression, EMT allows carcinoma cells to migrate, invade their local tissue environments and intravasate to the vasculature during metastasis [2]. During EMT, epithelial cells lose their cell polarity and cell–cell contacts with reorganization of actin cytoskeleton from a cortical structure to a stress fiber. Concomitantly, epithelial cells down-regulate epithelial-specific proteins, such as E-cadherin and cytokeratins, and induce mesenchymal proteins including N-cadherin, vimentin, and α-smooth muscle actin (α-SMA). Consequently, these cells can digest and migrate through the extracellular matrix [1,2].

Transforming growth factor-β (TGF-β) is known as a major inducer of EMT [3], and is also known to promote tumor cell invasiveness and metastasis in later stages of tumorigenesis [4]. Tumor cells often oversecrete TGF-β, which autonomously increases the motility and invasiveness of tumor cells especially through the induction of EMT, resulting in enhanced metastasis and poor prognosis [3,4]. TGF-β elicits its cellular response through type I and type II receptor serine/threonine kinases (TβRI and TβRII, respectively) [4]. Upon TGF-β binding, constitutively active TβRII transactivates TβRI serine/threonine kinase. Activated TβRI phosphorylates receptor-regulated Smads (Samd2 and Smad3), which then bind to common mediator Smad4, and accumulate in the nucleus where they regulate transcription of target genes [4].

Epithin, also known as suppression of tumorigenecity 14 (ST14), matriptase and membrane-type serine protease-1 (MT-SP1), is a type II transmembrane serine protease expressed in almost all normal and malignant epithelial tissues [5,6]. The proteolytic activity of epithin is strictly controlled by a Kunitz-type serine protease inhibitor, hepatocyte growth factor activator inhibitor 1 (HAI1), which binds to active epithin tightly and blocks the protease activity of epithin [7]. Studies on the epithin-deficient mice have revealed that epithin has physiological functions in terminal differentiation of epidermis, hair follicle development and thymic homeostasis [8]. Interestingly, epithin is consistently overexpressed in epithelial tumors when compared to corresponding normal tissues, and an elevated level of epithin correlates with the grade of malignancy and a poor patient outcome [9]. Moreover, strong evidences have been accumulated that increased level of epithin promotes tumorigenesis, tumor growth, and metastasis. Transgenic mice with increased expression of epithin in the skin demonstrated that overexpression of epithin was sufficient to induce squamous

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cell carcinoma and strongly potentiated chemical-induced tumor formation [10]. And also, cancer cells with increased level of epithin showed larger tumor mass, enhanced tumor angiogenesis, and accelerated dissemination when implanted into immunodeficient mice [11,12]. Conversely, down-regulation of epithin or inhibition of its protease activity using specific inhibitors has been reported to cause reduced tumor size and impaired metastasis [13,14]. Although upregulated epithin expression tightly correlates with cancer promotion, there is no report so far on the mechanism of epithin upregulation in the context of tumor progression.

In this study, we aimed to find the mechanism underlying the tumor-promoting effects of epithin, and based on this finding, to reveal the signaling pathway involved in epithin upregulation. Here, we show that epithin is transcriptionally upregulated by TGF-β and that this upregulation of epithin is essential for mediating TGF-β-induced EMT, thereby enhancing cell migration and invasion.

2. Materials and methods

2.1. Cell lines and reagents

Mardin–Darby canine kidney (MDCK) cells were maintained in DMEM (GIBCO) supplemented with 10% certified fetal bovine serum (GIBCO). 427.1.86 cells were maintained as previously described [15,16]. TGF-β was obtained from R&D systems.

2.2. Plasmids and siRNA

pcDNA3/epithin was previously described [15,16]. pGL3-epithin promoter was generated by PCR of 1 kb fragment of the 5′ flanking region of mouse epithin gene and cloning into pGL3-Promoter Vector (Promega). To generate pSUPER-epi, two synthetic oligonucleotides (forward strand: 5′-GATCCCC**GGTGCGCTTCAAACTCTTC**TTCAAGAGA**GAAGAGTTTGAAGCGCACC**TTTTTA-3′, reverse strand: 5′-TCGATAAAAA**GGTGCGCTTCAAACTCTTC**TCTCTTGAA**GAAGAGTTTGAAGCGCACC**GGG-3′, bold: target sequence) were annealed, and cloned into pSUPER vector (OligoEngine). To generate pSUPER-control, target was replaced with a scrambled sequence (5′-**GTGCGCGTTACAATCTCTC**-3′). To generate epithin-overexpressing clones, MDCK cells were transfected with pcDNA3/epithin, and were maintained in the presence of 400 μg/ml G418 for 10 days. To establish epithin knockdown cell lines, 427.1.86 cells were transfected with pSUPER-epi along with pcDNA3.1 that encodes a neomycin resistant gene, and were maintained in the presence of 800 μg/ml G418 for 10 days. Control clones were obtained after transfecting pSUPER-control. Control siRNA and siRNA for Smad2, Smad3, and Smad4 were purchased from Santa Cruz Biotechnologies. Cells were transfected using Lipofectamine 2000 or Lipofectamine PLUS reagent according to the manufacturer's instructions (Invitrogen).

2.3. Immunoblotting

Cells were lysed in Laemmli SDS reducing buffer, boiled and subjected to SDS–PAGE. Following antibodies were used: anti-epithin monoclonal antibody described previously [15,16], anti-epithin/matriptase polyclonal antibody (Calbiochem), anti-Smad4 (Santa Cruz), anti-Smad2/3 (Santa Cruz), anti-β-tubulin (Sigma), anti-HAI1 (Santa Cruz), anti-N-cadherin (BD transduction laboratories), anti-E-cadherin (BD transduction laboratories), anti-α-SMA (Chemicon), and anti-GAPDH (Santa Cruz) antibodies.

2.4. Quantitative real-time RT-PCR

Total RNA was prepared using TRIZOL reagent (Invitrogen), and reverse transcribed with SuperScript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Real-time PCR was performed on Applied Biosystems 7300 Real-time PCR system using Taqman universal Master Mix (Applied Biosystems). Specific primers and probe for epithin: forward (5′-TCATCGCCTACTACTGGTCAGAGT-3′), reverse (5′-TGGCGCGATCAACCTCTT-3′) and probe (5′-FAM-CAGCATCCCCCCACACCTGGC-TAMRA-3′); GAPDH: forward (5′-TGGCCTCCAAGGAGTAAGAAAC-3′), reverse (5′-GGGATAGGGCCTCTCTTGCT-3′) and probe (5′-FAM-CTGGACCACCCACCCCAGCAA-TAMRA-3′).

2.5. Luciferase reporter assay

Cells were transfected with pGL3-epithin promoter along with the pCMV-β-galactosidase vector (Clontech) as an internal control plasmid. The assay was performed as directed by the manufacturer (Promega), and the luciferase activity was detected with a FLUOstar OPTIMA (BMG LABTECH). The β-galactosidase assay was performed with the β-Galactosidase Enzyme Assay System (Promega).

2.6. Immunocytochemistry

Immunocytochemistry was performed as described before [16] using anti-E-cadherin antibody and affinity-purified anti-epithin polyclonal antibody. As a secondary antibody, FITC-labeled anti-mouse IgG or Cy5-labeled anti-mouse IgG for E-cadherin, and FITC-labeled anti-rabbit IgG for epithin were used (Jackson ImmunoResearch). For visualizing actin cytoskeleton, cells were stained with Rhodamine-conjugated Phalloidin (Molecular Probes). Cells were observed under an epi-fluorescence microscope, Axiovert 200M or a confocal laser scanning microscope, LSM510 (Carl Zeiss). Individual images were processed with Photoshop CS (Adobe System).

2.7. Wound healing assay

Cells were grown to form a confluent monolayer. The monolayer was scratched using a pipette tip, and then incubated in 0.5% serum-containing media for 20 h. Photographs were taken at 0 and 20 h after wounding using Axiovert 200M.

2.8. Transwell migration assay and invasion assay

The migration assay or invasion assay was performed using Transwell plates (8 μm pore size, Corning Incorporated) or BD BioCoat Matrigel Invasion Chamber (BD Biosciences) according to the manufacturer's instructions, respectively. After cells were cultured in the growth media with or without TGF-β for 2 days, 2×10^4 cells for migration assay or 10×10^4 cells for invasion assay were seeded in a serum-free medium into the upper chamber. The lower chamber was filled with DMEM containing 2% serum with or without TGF-β. After 18 h of incubation, cells were fixed, and the cells on the upper surface of the membrane were removed using cotton swabs. Migrated or invaded cells on the lower surface of the membrane were stained with DAPI (Molecular Probes), and counted under Axiovert 200M.

3. Results

3.1. Epithin overexpression induces EMT

To examine the effects of epithin on EMT, we established stable MDCK clones expressing mouse epithin; epi-F8 and epi-G6 (Fig. 1A). Interestingly, overexpression of epithin caused considerable morphological changes of MDCK cells from polarized epithelial morphology to an elongated fibroblast-like morphology (Fig. 1B). Immunocytochemical staining for E-cadherin and actin

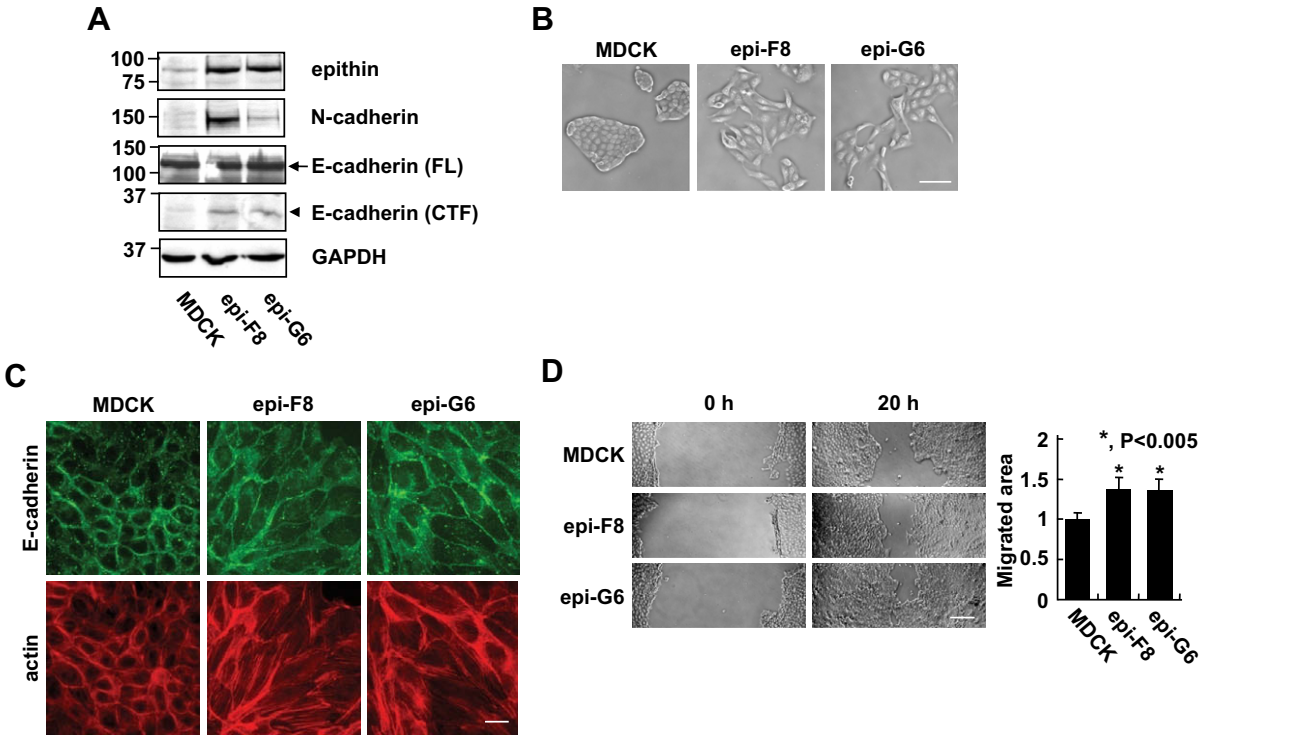

Fig. 1. Forced overexpression of epithin induces EMT of MDCK cells. (A) The expression of EMT-related proteins in parental MDCK cells and epithin-overexpressing MDCK clones (epi-F8 and epi-G6) was examined by immunoblotting with anti-epithin/matriptase polyclonal and anti-N-cadherin, E-cadherin, GAPDH antibodies. Full-length (FL) and C-terminal fragment (CTF) of E-cadherin are indicated by arrow and arrowhead, respectively. (B) Phase-contrast images of parental MDCK, epi-F8, and epi-G6 cells. Scale bar represents 100 μm. (C) The adherent junctions and actin cytoskeleton of MDCK, epi-F8, and epi-G6 cells were observed by staining for E-cadherin and actin filaments. Scale bar represents 20 μm. (D) At 0 and 20 h after wounding of MDCK, epi-F8, and epi-G6 cells, the wound area was observed by phase-contrast microscopy. Scale bar represents 200 μm. The graph represents the quantification of the migrated area of cells into a defined wound after 20 h. Data are presented as mean ± SD from three independent experiments.

cytoskeleton showed that elongated morphology of epithin-overexpressing cells was accompanied by the disassembly of cell–cell junctions and the formation of actin stress fibers (Fig. 1C). To verify that these morphological changes were associated with EMT, we examined the expression of EMT-related markers. In epithin-overexpressing clones, upregulation of N-cadherin, a mesenchymal marker, was detected and the generation of C-terminal fragment of E-cadherin was substantially increased (Fig. 1A). Proteolytic cleavage of E-cadherin has been considered to promote the disassembly of cell–cell junctions [17,18], suggesting that cleavage of E-cadherin induced by epithin overexpression could contribute to the disruption of cell–cell contacts observed in Fig. 1C. Since cells undergoing EMT acquire a migratory phenotype [1,2], we tested the effects of epithin overexpression on cell motility. Epithin overexpression significantly enhanced migration of MDCK cells (Fig. 1D). Collectively, these data show that increased expression of epithin causes EMT of epithelial cells.

3.2. TGF-β induces EMT concomitant with epithin upregulation

Since TGF-β has been implicated as a major inducer of EMT [3], we examined the effects of TGF-β on the expression of epithin in MDCK cells undergoing EMT. When stimulated with TGF-β for 3 days, MDCK cells showed the dissolution of cell–cell junctions, as marked by a significant dislocation of E-cadherin from cell–cell contacts, as well as the rearrangement of cortical actin cytoskeleton into stress fibers along with the acquisition of an elongated morphology (Fig. 2A). N-cadherin and α-SMA, mesenchymal proteins, were upregulated and C-terminal fragment of E-cadherin was generated upon TGF-β treatment (Fig. 2B). Notably, the expression of epithin was upregulated upon TGF-β stimulation (Fig. 2B). To confirm that epithin is upregulated in TGF-β-induced EMT of epithelial cells besides MDCK cells, we examined the effects of TGF-β on 427.1.86 cells. 427.1.86 is a thymic epithelial cell line derived from SV40 T-antigen transgenic mice, which constitutively expresses epithin [15,16,19]. TGF-β stimulation induced EMT of 427.1.86 cells to exhibit morphological changes including the disruption of cell–cell contacts and the rearrangement of actin filaments (Fig. 2C) and alternations in EMT-related proteins including N-cadherin and E-cadherin (Fig. 2D). Upregulation of epithin expression was also detected upon TGF-β stimulation (Fig. 2D). These experiments demonstrate that TGF-β induces EMT, paralleled by an increase in epithin level.

3.3. Epithin is critical for EMT induced by TGF-β

Epithin overexpression is analogous to TGF-β stimulation for the induction of EMT, suggesting that upregulated epithin upon TGF-β stimulation has an essential role in mediating EMT. To verify this possibility, we generated 427.1.86 clones stably expressing shRNA for epithin; Epi-KD-01 and Epi-KD-02. Epithin expression was attenuated in epithin knockdown cell lines compared to parental 427.1.86 cells and control cell lines (Fig. 3A). The expression of N-cadherin and α-SMA was also down-regulated in epithin knockdown cell lines in both conditions with or without TGF-β (Fig. 3A). This result indicates that both basal and TGF-β-induced expression of mesenchymal proteins correlate with the level of epithin expression. After 2 days of stimulation with TGF-β, 427.1.86 cells showed dislocation of E-cadherin from adherent junctions and rearrangement of actin cytoskeleton (Fig. 3B, middle panels). In contrast, Epi-KD-01 cells were resistant to the dissolution of adherent junctions in response to TGF-β and also were

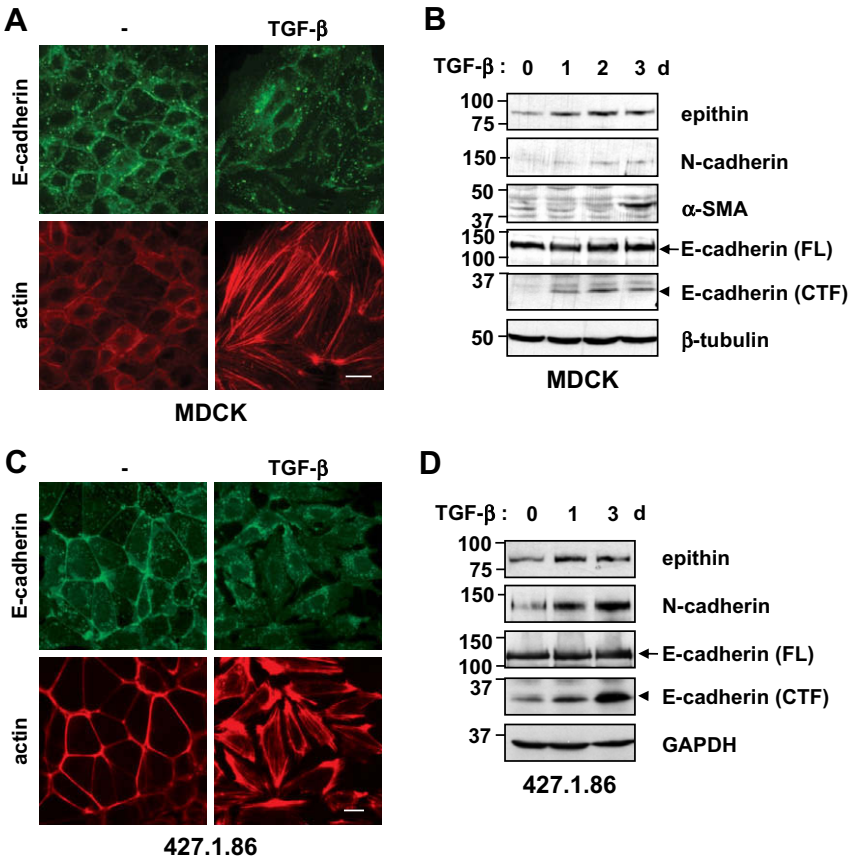

Fig. 2. Epithin is upregulated in TGF-β-induced EMT. (A and C) MDCK cells (A) and 427.1.86 cells (C) were cultured in the absence or presence of TGF-β (5 ng/ml) for 3 days, and were stained for E-cadherin and actin filaments. Scale bar represents 20 μm. (B and D) MDCK cells (B) and 427.1.86 cells (D) were stimulated with TGF-β for the indicated time, and the lysates were analyzed by immunoblotting using anti-epithin/matriptase polyclonal antibody and antibodies against N-cadherin, E-cadherin, α-SMA, and β-tubulin (B) and using anti-epithin, N-cadherin, E-cadherin, and GAPDH antibodies (D). Full-length (FL) and C-terminal fragment (CTF) of E-cadherin are indicated by arrows and arrowheads, respectively.

resistant to TGF-β-induced actin cytoskeleton rearrangement, so that most of them retained cortical actin staining even with TGF-β treatment (Fig. 3B, right panels). As a consequence of EMT, epithelial cells acquire the ability to migrate and invade into the extracellular matrix [1,2]. Accordingly, we attempted to evaluate the role of epithin in TGF-β-induced cell migration and invasion. 427.1.86 cells stimulated by TGF-β showed significantly enhanced motility in the two-chamber migration assay (Fig. 3C). However, TGF-β-induced cell migration was severely impaired in both Epi-KD-01 and Epi-KD-02 cells (Fig. 3C). TGF-β also enhanced the invasion of 427.1.86 cells through Matrigel by 4-fold, whereas in epithin knockdown cell lines TGF-β-mediated invasion was strongly attenuated (Fig. 3D). Taken together, these results suggest that epithin plays an important role in mediating TGF-β-induced EMT, thereby enhancing cell migration and invasion.

3.4. TGF-β-induced epithin upregulation is mediated by Smad-dependent transcriptional activation

To investigate whether the Smad-dependent pathway is involved in TGF-β-induced epithin upregulation, we introduced siRNA targeting the common Smad mediator, Smad4. Smad4 siRNA effectively inhibited Smad4 expression, and Smad4 knockdown completely abolished epithin mRNA induction by TGF-β stimulation (Fig. 4A). In addition, knockdown of Smad2, but not Smad3 efficiently inhibited TGF-β-induced epithin expression (Fig. 4B). These data suggest that Smad2/Smad4 complex plays a role in regulation of epithin expression. To investigate the mechanism of epithin induction by TGF-β, we tested the effect of TGF-β on the activity of epithin promoter. As luciferase activity was increased upon TGF-β treatment (Fig. 4C), we concluded that TGF-β upregulates epithin via transcriptional activation. Additionally, we tested whether HAI1, a cognate inhibitor of epithin, was regulated by TGF-β. TGF-β upregulated epithin without an increase in HAI1 protein (Fig. 4D), indicating that increased epithin by TGF-β could evade HAI1-mediated inhibition of its proteolytic activity.

4. Discussion

Here, we unveiled that TGF-β signaling is involved in the regulation of epithin expression. To our knowledge, this is the first finding of the upregulation of epithin expression at a transcriptional level. Interestingly, it has been reported that epithin is substantially down-regulated by bikunin; a Kunitz-type protease inhibitor, which is known to suppress tumor cell invasion and metastasis [20]. Subsequently, bikunin has been reported to disrupt ligand-induced oligomerization of TGF-β receptors, thereby suppressing TGF-β signaling [21]. Combining these findings and our results, we suggest that bikunin may down-regulate the expression of epithin by suppressing TGF-β-elicited signaling.

In our study, TGF-β only modestly increased the expression of epithin 1.3- to 1.5-fold. Previously, it has been reported that even a modest overexpression of epithin mRNA (1.2- to 1.4-fold) in the skin of transgenic mice is sufficient to initiate malignant transformation and to potentiate carcinogen-induced tumorigenesis [10]. Surprisingly, transgenic expression of HAI1 in the epidermis

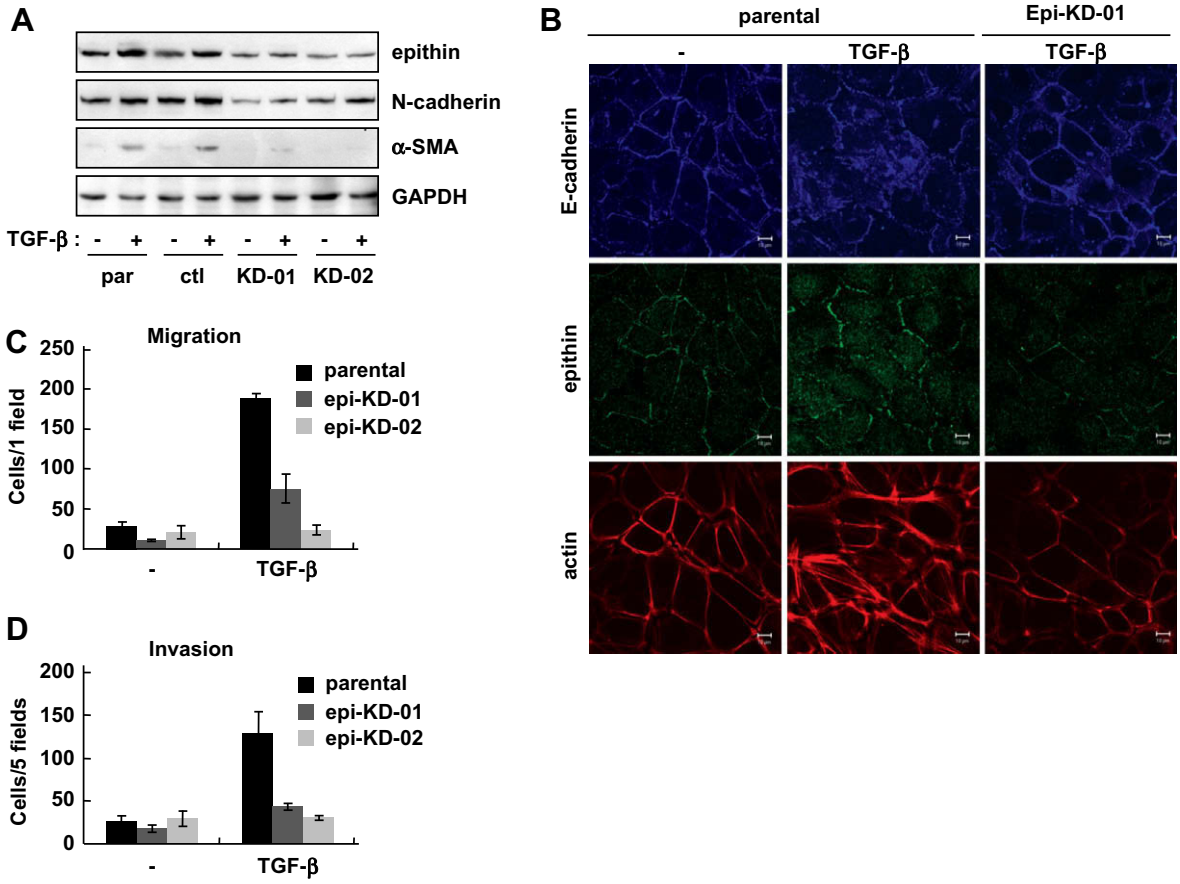

Fig. 3. Knockdown of epithin inhibits TGF-β-induced EMT and cell invasion. (A) 427.1.86 cells (par) and cell lines stably expressing pSUPER-control (ctl) and pSUPER-epi (KD-01 and KD-02) were cultured without or with TGF-β (5 ng/ml) for 2 days. Immunoblotting was performed against epithin, N-cadherin, α-SMA, and GAPDH. (B) 427.1.86 and Epi-KD-01 cells were cultured without or with TGF-β for 2 days, and then were stained for E-cadherin, epithin, and actin filaments. Scale bar represents 10 μm. (C and D) 427.1.86, Epi-KD-01 and Epi-KD-02 cells were cultured without or with TGF-β for 2 days. Cells (2×10^4) were seeded on the upper side of Transwell plate (C) or 10×10^4 cells were seeded on the upper side of BioCoat Matrigel invasion chamber (D). After 20 h, cells on the bottom side of membrane were fixed, stained and counted in five random fields. Data are representative for three independent experiments and shown as mean ± SD.

completely negated malignant transformation and progression in epithin transgenic mice [10]. Thus, deregulated epithin unopposed by HAI1 possesses a strong oncogenic potential. Consistent with these observations, it has been reported that increased expression of epithin relative to HAI1 correlates with the grade of malignancy in human cancers [22,23]. Since the level of HAI1 expression was not altered by TGF-β stimulation (Fig. 4D), TGF-β can perturb the balance between epithin and HAI1 toward epithin. Thus, our expectation is that TGF-β increases deregulated epithin unopposed by HAI1, which contributes to TGF-β-induced EMT. This is further supported by the fact that loss of HAI1 caused the disruption of epithelial integrity, which was fully rescued by simultaneous loss of epithin in mice and zebrafish models [24,25]. These reports indicate that HAI1 contributes to retain epithelial cell characteristics by inhibiting epithin. Recently, it has been reported that knockdown of HAI1 in human cancer cell lines induced EMT, which was partially reversed by concurrent silencing of epithin [26], indicating that deregulated activities of epithin in concert with other target proteases of HAI1 caused EMT of epithelial-derived cancer cells.

Our results show that epithin is crucial for TGF-β-induced EMT, but the underlying mechanism is not fully understood. One possibility is that upregulated epithin proteolytically activates its substrates, which promotes EMT. Indeed, evidence has accumulated indicating that some epithin substrates, such as urokinase type plasminogen activator (uPA), HGF/SF, macrophage stimulating protein-1 (MSP-1) and matrix metalloproteinase-3 (MMP-3), are implicated in EMT [27–30]. Notably, MMP-3/stromelysin-1 has been reported to induce EMT in mouse mammary epithelial cells and mouse skin tumor cells [30,31]. Furthermore, constitutively active MMP-3 promotes the development of premalignant and malignant mammary lesions in transgenic mice [32]. This is intriguingly analogous to the phenotype of transgenic mice with overexpression of epithin in the skin [10], and epithin can proteolytically activate MMP-3 [12]. In addition, MMP-3 triggered the cleavage of E-cadherin accompanied by the dissolution of cell–cell junctions [30]. Likewise, epithin overexpression caused the generation of truncated E-cadherin (Fig. 1A), which was also accompanied by the dissolution of cell–cell contacts (Fig. 1C). Thus, the molecular pathway exerted by MMP-3 is likely to be related to epithin in mediating EMT. Another expectation is that epithin proteolytically activates the latent TGF-β complex as do other serine proteases [33]. Epithin overexpression mimicked TGF-β stimulation for mediating EMT (Figs. 1 and 2), which supports this possibility. According to this hypothesis, upregulated epithin activates latent TGF-β, leading to the establishment of autocrine TGF-β signaling, resulting in further production of epithin and subsequent amplification.

In conclusion, we show that TGF-β upregulates epithin expression via Smad2/Smad4-dependent transcriptional activation, and that upregulated epithin has a pivotal role in mediating EMT induced by TGF-β. These findings may contribute to a better under-

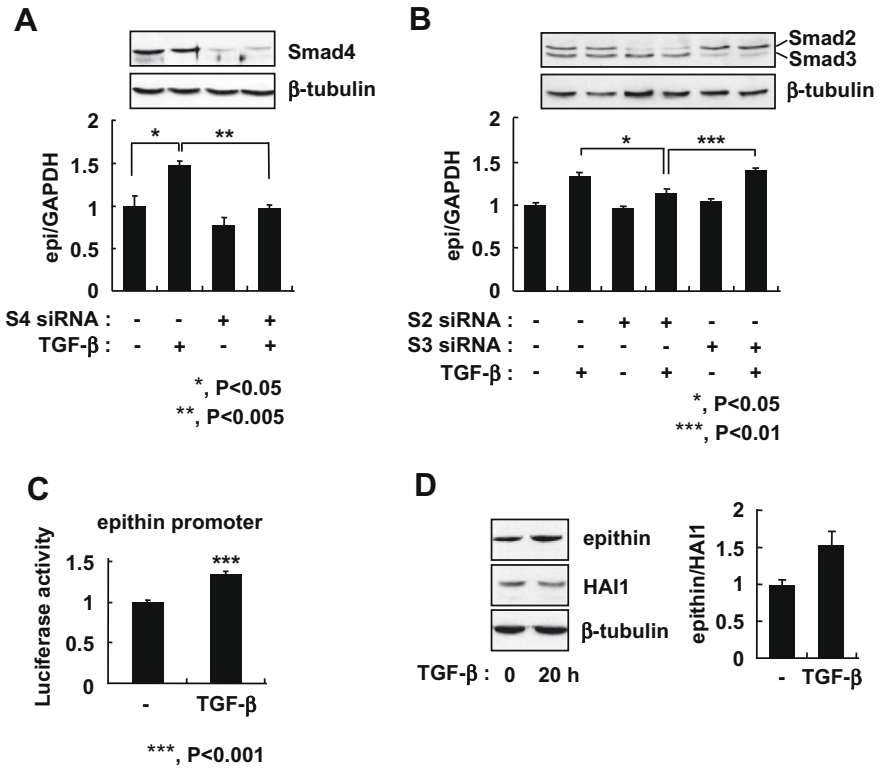

Fig. 4. TGF-β-induced epithin upregulation is mediated by Smad-dependent transcriptional activation. (A and B) 427.1.86 cells were transfected with siRNA targeting Smad4 (A), Smad2 or Smad3 (B), and after 1 day, stimulated with TGF-β (5 ng/ml) for 20 h. Knockdown of each Smad protein was confirmed by immunoblotting. Relative epithin mRNA to GAPDH mRNA was analyzed by real-time PCR. (C) Cells were transfected with pGL3-epithin promoter vector. After 2 days, TGF-β was treated for 20 h, and luciferase activity was measured. The results are shown as relative luciferase activity normalized to β-gal activity. (D) Cells were stimulated with TGF-β for 20 h, and the levels of epithin and HAI1 were assessed by immunoblotting. The graph represents the ratio of epithin to HAI1. Data in the bar graphs represent the mean ± SD from three independent experiments.

standing of the molecular mechanisms underlying EMT and tumor progression.

Acknowledgments

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References

[1] J. Yang, R.A. Weinberg, Epithelial–mesenchymal transition: at the crossroads of development and tumor metastasis, Dev. Cell 14 (2008) 818–829.
[2] J.P. Thiery, Epithelial–mesenchymal transitions in tumour progression, Nat. Rev. Cancer 2 (2002) 442–454.
[3] J. Xu, S. Lamouille, R. Derynck, TGF-β-induced epithelial to mesenchymal transition, Cell Res. 19 (2009) 156–172.
[4] J. Massague, TGFβ in cancer, Cell 134 (2008) 215–230.
[5] S. Netzel-Arnett, J.D. Hooper, R. Szabo, E.L. Madison, J.P. Quigley, T.H. Bugge, T.M. Antalis, Membrane anchored serine proteases: a rapidly expanding group of cell surface proteolytic enzymes with potential roles in cancer, Cancer Metastasis Rev. 22 (2003) 237–258.
[6] M. Oberst, J. Anders, B. Xie, B. Singh, M. Ossandon, M. Johnson, R.B. Dickson, C.Y. Lin, Matriptase and HAI-1 are expressed by normal and malignant epithelial cells in vitro and in vivo, Am. J. Pathol. 158 (2001) 1301–1311.
[7] K. List, T.H. Bugge, R. Szabo, Matriptase: potent proteolysis on the cell surface, Mol. Med. 12 (2006) 1–7.
[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. Uhland, Matriptase and its putative role in cancer, Cell. Mol. Life Sci. 63 (2006) 2968–2978.
[10] 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.
[11] S. Ihara, E. Miyoshi, J.H. Ko, K. Murata, S. Nakahara, K. Honke, R.B. Dickson, C.Y. Lin, N. Taniguchi, Prometastatic effect of *N*-acetylglucosaminyltransferase V is due to modification and stabilization of active matriptase by adding β 1–6 GlcNAc branching, J. Biol. Chem. 277 (2002) 16960–16967.
[12] X. Jin, M. Yagi, N. Akiyama, T. Hirosaki, S. Higashi, C.Y. Lin, R.B. Dickson, H. Kitamura, K. Miyazaki, Matriptase activates stromelysin (MMP-3) and promotes tumor growth and angiogenesis, Cancer Sci. 97 (2006) 1327–1334.
[13] M. Suzuki, H. Kobayashi, N. Kanayama, Y. Saga, M. Suzuki, C.Y. Lin, R.B. Dickson, T. Terao, Inhibition of tumor invasion by genomic down-regulation of matriptase through suppression of activation of receptor-bound pro-urokinase, J. Biol. Chem. 279 (2004) 14899–14908.
[14] A.V. Galkin, L. Mullen, W.D. Fox, J. Brown, D. Duncan, O. Moreno, E.L. Madison, D.B. Agus, CVS-3983, a selective matriptase inhibitor, suppresses the growth of androgen independent prostate tumor xenografts, Prostate 61 (2004) 228–235.
[15] 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.
[16] 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.
[17] P. Marambaud, J. Shioi, G. Serban, A. Georgakopoulos, S. Sarner, V. Nagy, L. Baki, P. Wen, S. Efthimiopoulos, Z. Shao, T. Wisniewski, N.K. Robakis, A presenilin-1/γ-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions, EMBO J. 21 (2002) 1948–1956.
[18] T. Maretzky, K. Reiss, A. Ludwig, J. Buchholz, F. Scholz, E. Proksch, B. de Strooper, D. Hartmann, P. Saftig, ADAM10 mediates E-cadherin shedding and regulates epithelial cell–cell adhesion, migration, and beta-catenin translocation, Proc. Natl. Acad. Sci. USA 102 (2005) 9182–9187.
[19] M.G. Kim, C. Chen, M.S. Lyu, E.G. Cho, D. Park, C. Kozak, R.H. Schwartz, Cloning and chromosomal mapping of a gene isolated from thymic stromal cells encoding a new mouse type II membrane serine protease, epithin, containing four LDL receptor modules and two CUB domains, Immunogenetics 49 (1999) 420–428.

[20] M. Suzuki, H. Kobayashi, Y. Tanaka, Y. Hirashima, N. Kanayama, Y. Takei, Y. Saga, M. Suzuki, H. Itoh, T. Terao, Bikunin target genes in ovarian cancer cells identified by microarray analysis, J. Biol. Chem. 278 (2003) 14640–14646.
[21] T. Yagyu, H. Kobayashi, K. Wakahara, H. Matsuzaki, T. Kondo, N. Kurita, H. Sekino, K. Inagaki, M. Suzuki, N. Kanayama, T. Terao, A kunitz-type protease inhibitor bikunin disrupts ligand-induced oligomerization of receptors for transforming growth factor (TGF)-β and subsequently suppresses TGF-β signalings, FEBS Lett. 576 (2004) 408–416.
[22] M.D. Oberst, M.D. Johnson, R.B. Dickson, C.Y. Lin, B. Singh, M. Stewart, A. Williams, A. al-Nafussi, J.F. Smyth, H. Gabra, G.C. Sellar, Expression of the serine protease matriptase and its inhibitor HAI-1 in epithelial ovarian cancer: correlation with clinical outcome and tumor clinicopathological parameters, Clin. Cancer Res. 8 (2002) 1101–1107.
[23] L.K. Vogel, M. Saebo, C.F. Skjelbred, K. Abell, E.D. Pedersen, U. Vogel, E.H. Kure, The ratio of matriptase/HAI-1 mRNA is higher in colorectal cancer adenomas and carcinomas than corresponding tissue from control individuals, BMC Cancer 6 (2006) 176.
[24] T.J. Carney, S. von der Hardt, C. Sonntag, A. Amsterdam, J. Topczewski, N. Hopkins, M. Hammerschmidt, Inactivation of serine protease matriptase1a by its inhibitor Hai1 is required for epithelial integrity of the zebrafish epidermis, Development 134 (2007) 3461–3471.
[25] R. Szabo, A. Molinolo, K. List, T.H. Bugge, Matriptase inhibition by hepatocyte growth factor activator inhibitor-1 is essential for placental development, Oncogene 26 (2007) 1546–1556.
[26] H. Cheng, T. Fukushima, N. Takahashi, H. Tanaka, H. Kataoka, Hepatocyte growth factor activator inhibitor type 1 regulates epithelial to mesenchymal transition through membrane-bound serine proteinases, Cancer Res. 69 (2009) 1828–1835.
[27] J.F. Santibanez, JNK mediates TGF-β1-induced epithelial mesenchymal transdifferentiation of mouse transformed keratinocytes, FEBS Lett. 580 (2006) 5385–5391.
[28] S. Grotegut, D. von Schweinitz, G. Christofori, F. Lehembre, Hepatocyte growth factor induces cell scattering through MAPK/Egr-1-mediated upregulation of Snail, EMBO J. 25 (2006) 3534–3545.
[29] E.R. Camp, A. Yang, M.J. Gray, F. Fan, S.R. Hamilton, D.B. Evans, A.T. Hooper, D.S. Pereira, D.J. Hicklin, L.M. Ellis, Tyrosine kinase receptor RON in human pancreatic cancer: expression, function, and validation as a target, Cancer 109 (2007) 1030–1039.
[30] A. Lochter, S. Galosy, J. Muschler, N. Freedman, Z. Werb, M.J. Bissell, Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells, J. Cell Biol. 139 (1997) 1861–1872.
[31] D.L. Hulboy, L.M. Matrisian, H.C. Crawford, Loss of JunB activity enhances stromelysin 1 expression in a model of the epithelial-to-mesenchymal transition of mouse skin tumors, Mol. Cell. Biol. 21 (2001) 5478–5487.
[32] M.D. Sternlicht, A. Lochter, C.J. Sympson, B. Huey, J.P. Rougier, J.W. Gray, D. Pinkel, M.J. Bissell, Z. Werb, The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis, Cell 98 (1999) 137–146.
[33] G. Jenkins, The role of proteases in transforming growth factor-β activation, Int. J. Biochem. Cell Biol. 40 (2008) 1068–1078.