Physiology International 109 (2022) 2, 204–214
DOI: 10.1556/2060.2022.00006

AKADÉMIAI KIADÓ

Collagen type X alpha 1 promotes proliferation, invasion and epithelial-mesenchymal transition of cervical cancer through activation of TGF-β/Smad signaling

YANGYAN SUN[1], JING LING[1] and LU LIU[2*]

[1] Department of Gynecology, Jiangyin People's Hospital, Wuxi, Jiangsu Province, 214400, China

[2] Department of Pediatrics, Wuhan Third Hospital, Wuhan, Hubei Province, 432500, China

Received: January 11, 2022 • Revised manuscript received: February 21, 2022 • Accepted: March 11, 2022
Published online: May 18, 2022

ABSTRACT

Background: Collagen type X alpha 1 (COL10A1) belongs to the collagen family and constitutes the main component of the interstitial matrix. COL10A1 was found to be dysregulated in various cancers, and to participate in tumorigenesis. However, the role of COL10A1 in cervical cancer (CC) remains unclear. *Methods:* Expression of COL10A1 in CC cells and tissues was detected by western blot and qRT-PCR. CC cells were transfected with pcDNA-COL10A1 or si-COL10A1, and the effect of COL10A1 on cell proliferation of CC was assessed by MTT and colony formation assays. Cell metastasis was detected by wound healing and transwell assays. Western blot was applied to evaluate epithelial-mesenchymal transition. *Results:* COL10A1 was significantly elevated in CC tissues and cells ($P < 0.001$). Over-expression of COL10A1 increased cell viability of CC ($P < 0.001$), and enhanced the number of colonies ($P < 0.001$). However, knockdown of COL10A1 reduced the cell proliferation of CC ($P < 0.001$). Over-expression of COL10A1 also promoted cell migration ($P < 0.001$) and invasion ($P < 0.001$) of CC, whereas silencing of COL10A1 suppressed cell metastasis ($P < 0.001$). Protein level of E-cadherin in CC was reduced ($P < 0.05$), whereas N-cadherin and vimentin were enhanced by COL10A1 over-expression ($P < 0.001$). Silencing of COL10A1 reduced the protein level of TGF-β1 ($P < 0.01$), and down-regulated the phosphorylation of Smad2 and Smad3 in CC ($P < 0.001$). *Conclusion:* Down-regulation of COL10A1 suppressed cell proliferation, metastasis, and epithelial-mesenchymal transition of CC through inactivation of TGF-β/Smad signaling.

* Corresponding author. Department of Pediatrics, Wuhan Third Hospital, No. 216, Guanshan Avenue, Hongshan District, Wuhan, Hubei Province, 432500, China. Tel.: +86 027 65399457. E-mail: liulu12_dr@163.com

KEYWORDS

collagen type X alpha 1, cervical cancer, proliferation, invasion, epithelial-mesenchymal transition, TGF-β/Smad

INTRODUCTION

Cervical cancer (CC) is one of the most common causes of cancer-related death in women worldwide [1, 2]. Risk factors, such as poor hygiene, human papilloma virus history, and polygamous spouse, contribute to the progression of CC [3]. CC is generally diagnosed at an advanced stage due to insufficient early detection [4], and the 5-year survival rate remains low [5]. Therefore, therapeutic targets for CC are urgently needed for the effective prevention of the disease.

Collagen type X alpha 1 (COL10A1) is a member of the collagen family, and constitutes the main component of the interstitial matrix [6]. COL10A1 is elevated in various solid tumors, including gastric [7], breast [8], and colorectal [9] cancer, and is associated with poor prognosis. COL10A1 is regarded as a diagnostic or prognostic biomarker for tumors, and might be a potential target for tumors [10–13]. Moreover, COL10A1 is also involved in many key cellular processes, including cell proliferation, migration, and invasion [14]. However, the role of COL10A1 in CC has not been reported yet.

TGF-β is a cytokine essential for the regulation of cellular processes, and plays a key role in tumor initiation and progression [15]. TGF-β binds to the receptors TGFβR1 and TGFβR2, recruits and phosphorylates Smads, thus regulating the transcription of target genes involved in cell proliferation, metastasis and epithelial-mesenchymal transition (EMT) of tumors [15]. It has been reported that TGF-β/Smad pathway is activated in CC [16]. TGF-β induced cell metastasis and EMT in CC [17], and suppression of TGF-β signaling contributed to the inhibition of EMT in CC [18]. In addition, TGF-β1 has been reported to phosphorylate Smad2 to increase protein expression of COL10A1, and to promote the invasion and EMT of gastric cancer [19]. Therefore, we hypothesized that COL10A1 might be involved in TGF-β/Smad-mediated EMT in CC.

In this study, the effects of COL10A1 on cell proliferation, metastasis and EMT of CC were investigated. The underlying mechanism involved in COL10A1-mediated CC may provide a potential target for the prevention of CC.

MATERIALS AND METHODS

Cell culture and transfection

Human CC cells (C-33A, HeLa, CaSki and ME-180) and cervical epithelial cells (HCerEpiC) were acquired from Shanghai Institutes for Biological Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Invitrogen) at 37 °C. pcDNA3.1-COL10A1 and the negative control (pcDNA3.1), as well as siRNA targeting COL10A1 (si-COL10A1) and the negative control (siRNA-NC) were purchased from Genepharma (Shanghai, China). HeLa and C-33A cells were transfected with the plasmids or siRNAs using Lipofectamine® 3000 (Invitrogen). Cells were transfected with the same volume of RPMI-1640 medium, and regarded as control (NC).

qRT-PCR. Cells were lysed in TRIzol (Invitrogen) to isolate the RNAs. RNAs were then synthesized into cDNAs using the Multiscribe™ Reverse transcription Kit (Applied Biosystems, CA, USA), and the mRNA expression of COL10A1 was detected by the PreTaq II kit (Takara, Dalian, Liaoning, China) with following primers through the $2^{-\triangle\triangle Ct}$ method:

COL10A1:

Forward: 5′-ATGCTGCCACAAATACCCTTT-3′;

Reverse: 5′-GGTAGTGGGCCTTTTATGCCT-3′.

The mRNA expression was normalized to GAPDH:

Forward: 5′-TCAACGACCACTTTGTCAAGCAGAGT-3′;

Reverse: 5′-GCTGGTGGTCCAGGGGTCTTACT-3′

Cell viability and proliferation assays

HeLa and C-33A cells were seeded in 96-wells plates and transfected with the indicated plasmids or siRNAs, and cultured for 24, 48 or 72 h. Cells in each well were incubated with 10 μL MTT solution (Beyotime, Beijing, China) for another 4 h. Dimethyl sulfoxide was then added, and the absorbance at 450 nm was measured by microplate reader (Bio-Rad, Hercules, CA, USA). For cell proliferation assay, HeLa and C-33A cells with indicated transfections were seeded in 6-well plates, and cultured for 10 days. Cells were fixed in methanol, stained with crystal violet, and then measured under microscope (Olympus, Tokyo, Japan). The colony numbers were calculated by Image J v.1.46 (National Institutes of Health, Bethesda, MD, USA).

Cell migration and invasion assays

HeLa and C-33A cells with indicated transfections were seeded in 6-well plates, and then scratched using a 200 μL pipette tip in the middle of each well. Twenty-four hours later, the wound was observed under the microscope (Olympus), and the wound width was also calculated by Image J. For cell invasion assay, HeLa and C-33A cells with indicated transfections were plated into the upper champers of Transwell chambers (Corning Incorporated, Corning, NY, USA) in serum-free medium. To the lower chambers, medium with 20% fetal bovine serum was added. Twenty-four hours later, cells in the lower chamber were stained with crystal violet, and observed under the microscope (Olympus). The number of invasive cells was also calculated by Image J.

Western blot

Cells were lysed in RIPA buffer (Beyotime), and the protein concentration was calculated by the BCA Protein Assay Kit (Sigma-Aldrich, St Louis, MO, USA). Proteins (30 μg) were separated by 10% SDS-PAGE, and then transferred onto nitrocellulose membranes. The membranes were blocked in 5% skim milk, and incubated with specific antibodies: anti-COL10A1 and anti-GAPDH (1:1500, Abcam, Cambridge, UK), anti-E-cadherin and anti-N-cadherin (1:2500, Abcam), anti-Vimentin and anti-TGF-β1 (1:3000, Abcam), anti-Smad2 and anti-p-Smad2 (1:3500, Abcam), anti-Smad3 and anti-p-Smad3 (1:4000, Abcam). After washing with PBS, the membranes were probed with horseradish peroxidase-conjugated secondary antibody (1:5000, Abcam), and immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich).

Statistical analysis

All the data with at least triple replicates were expressed as mean ± SEM, and analyzed by student's *t*-test or one-way analysis of variance using SPSS software. A *P* value of <0.05 was considered as statistically significant.

RESULTS

COL10A1 was elevated in CC

Data based on the TCGA database showed that COL10A1 was significantly up-regulated in CC tissues ($n = 305$) compared to normal tissues ($n = 3$) (Fig. 1A). Human CC cells (C-33A, HeLa, CaSki and ME-180) also exhibited higher mRNA (Fig. 1B) and protein (Fig. 1C and D) expression than cervical epithelial cells (HCerEpiC), suggesting that COL10A1 might be involved in CC progression.

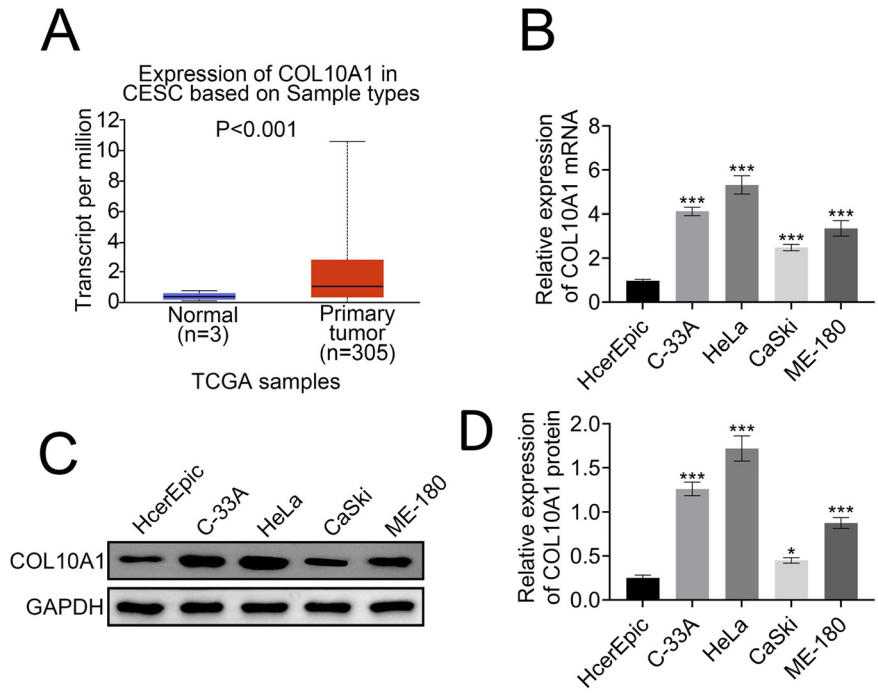

Fig. 1. COL10A1 was elevated in cervical cancer
(A) COL10A1 was up-regulated in CC tissues ($n = 305$) compared to the normal tissues ($n = 3$) based on the TCGA database. CESC: cervical squamous cell carcinoma. (B) COL10A1 mRNA was up-regulated in Human CC cells (C-33A, HeLa, CaSki and ME-180) compared to cervical epithelial cells (HCerEpiC). (C) COL10A1 protein was up-regulated in Human CC cells (C-33A, HeLa, CaSki and ME-180) compared to cervical epithelial cells (HCerEpiC). (D) Relative protein expression of COL10A1 in Human CC cells (C-33A, HeLa, CaSki and ME-180) and HcerEpiC. $n = 3$. * $P < 0.05$, *** $P < 0.001$

COL10A1 contributed to cell proliferation of CC

To assess the effects of COL10A1 on CC, HeLa and C-33A cells were transfected with pcDNA3.1-COL10A1 or si-COL10A1. Transfection with pcDNA3.1-COL10A1 or si-COL10A1 increased or decreased, respectively, the protein expression of COL10A1 (Fig. 2A and B). Over-expression of COL10A1 increased cell viability of HeLa and C-33A, whereas knockdown of COL10A1 decreased the cell viability (Fig. 2C). Moreover, COL10A1 over-expression

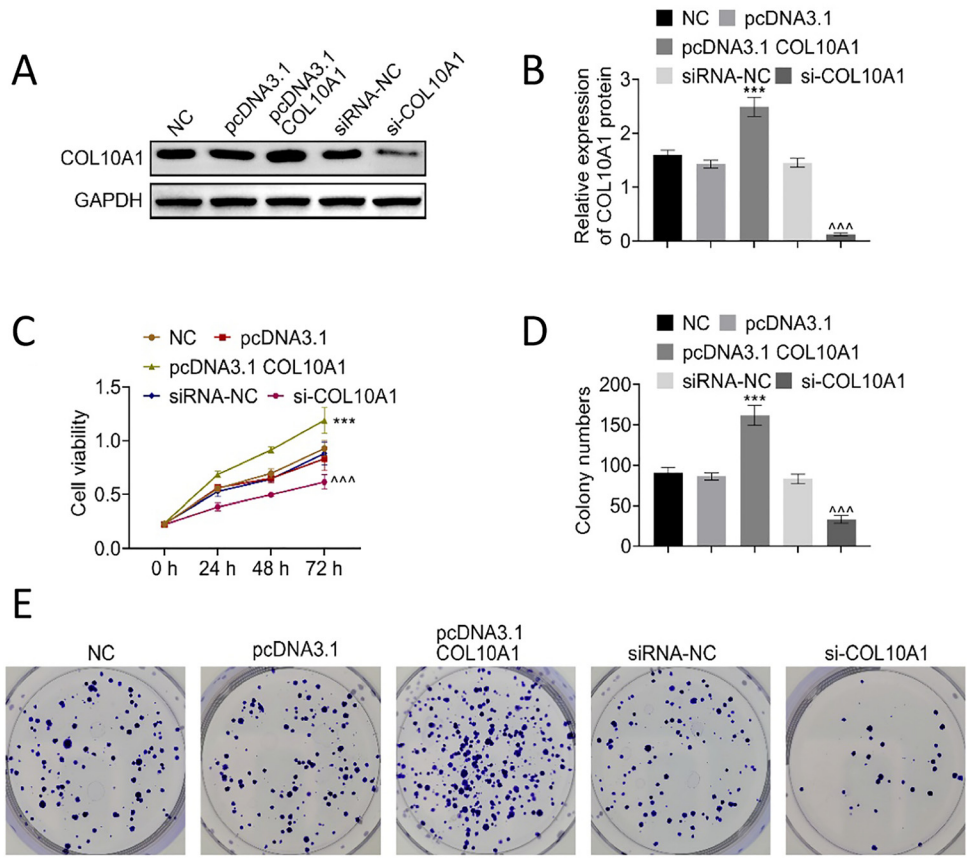

Fig. 2. COL10A1 contributed to cell proliferation of cervical cancer
(A) Transfection with pcDNA3.1-COL10A1 or si-COL10A1 increased or decreased protein expression of COL10A1, respectively in Hela and C-33A. (B) Relative protein expression of COL10A1 in HeLa and C-33A transfected with pcDNA3.1-COL10A1 or si-COL10A1. (C) Over-expression of COL10A1 increased cell viability of HeLa and C-33A, whereas knockdown of COL10A1 decreased the cell viability. (D) Over-expression of COL10A1 increased cell proliferation of HeLa and C-33A, whereas knockdown of COL10A1 decreased the cell proliferation. (E) Over-expression of COL10A1 increased colony number of HeLa and C-33A, whereas knockdown of COL10A1 decreased the colony number. $n = 3$. *** vs. pcDNA3.1, $P < 0.001$. ^^^ vs. siRNA-NC, $P < 0.001$

promoted cell proliferation of HeLa and C-33A (Fig. 2D) through enhancing of colony number (Fig. 2E), whereas COL10A1 silencing suppressed proliferation (Fig. 2D) through reduction of colony number (Fig. 2E). These results suggested the anti-proliferative effect of COL10A1 silencing on CC.

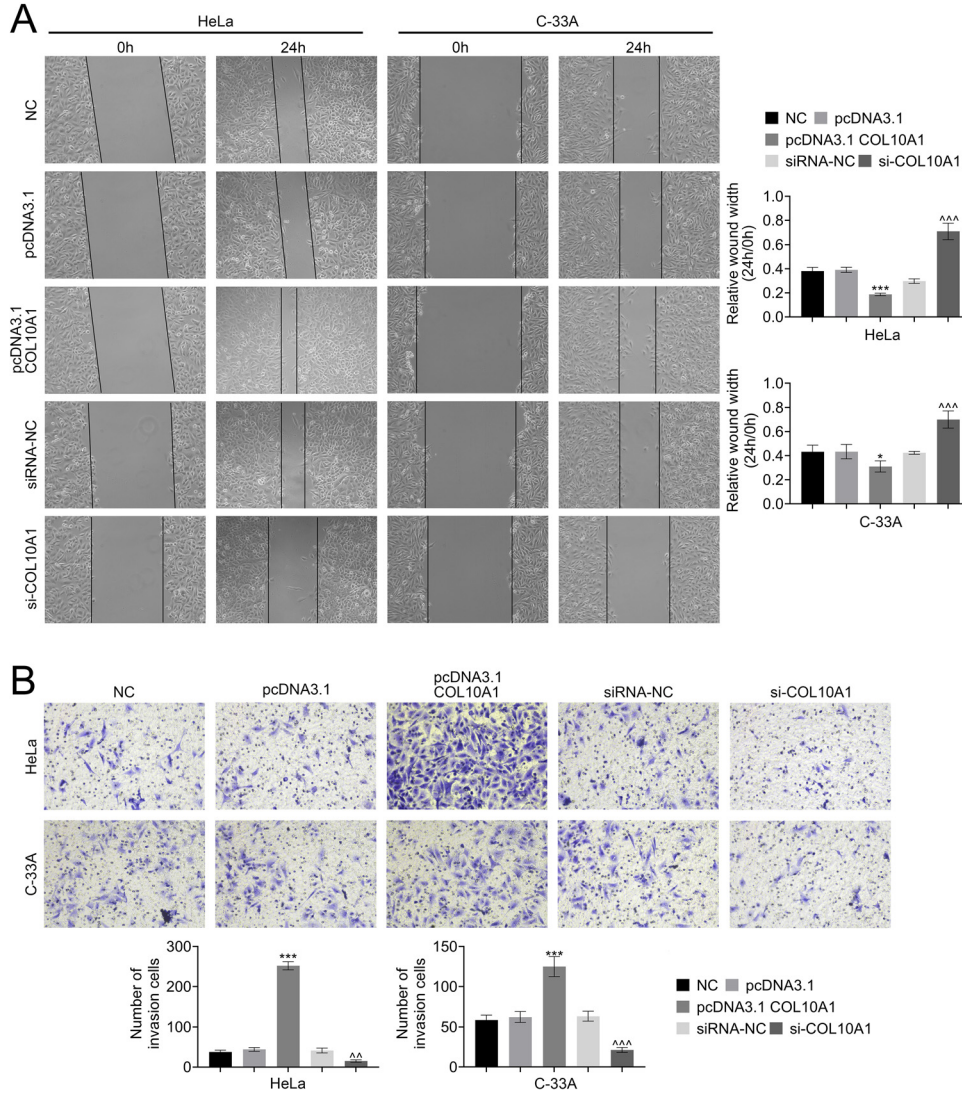

Fig. 3. COL10A1 contributed to cell metastasis of cervical cancer

(A) Over-expression of COL10A1 increased wound width of HeLa and C-33A, whereas knockdown of COL10A1 decreased the wound width. (B) Over-expression of COL10A1 promoted cell invasion of HeLa and C-33A, whereas knockdown of COL10A1 suppressed the cell invasion. $n = 3$. *** vs. pcDNA3.1, $P < 0.001$. ^^, ^^^ vs. siRNA-NC, $P < 0.01$, $P < 0.001$

COL10A1 contributed to cell metastasis of CC

Cell migration of HeLa and C-33A cells was promoted by over-expression of COL10A1 (Fig. 3A), whereas silencing of COL10A1 repressed cell migration (Fig. 3A). Similarly, transfection with pcDNA3.1-COL10A1 promoted cell invasion of HeLa and C-33A cells with increased number of invasive cells (Fig. 3B). However, cell invasion of HeLa and C-33A cells

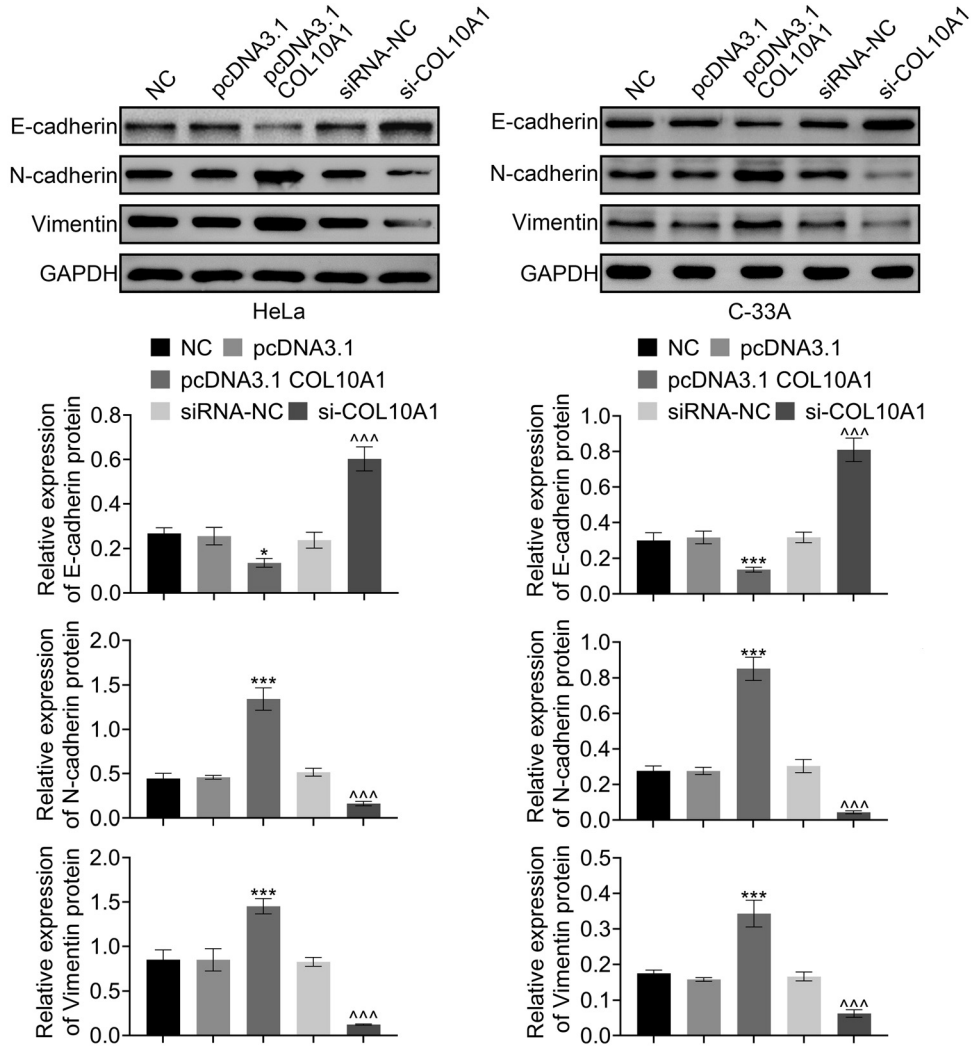

Fig. 4. COL10A1 contributed to EMT of cervical cancer
Over-expression of COL10A1 reduced protein expression of E-cadherin, enhanced N-cadherin and Vimentin in HeLa and C-33A, whereas knockdown of COL10A1 enhanced E-cadherin, reduced N-cadherin and Vimentin. $n = 3$. *** vs. pcDNA3.1, $P < 0.001$. ^^^ vs. siRNA-NC, $P < 0.001$

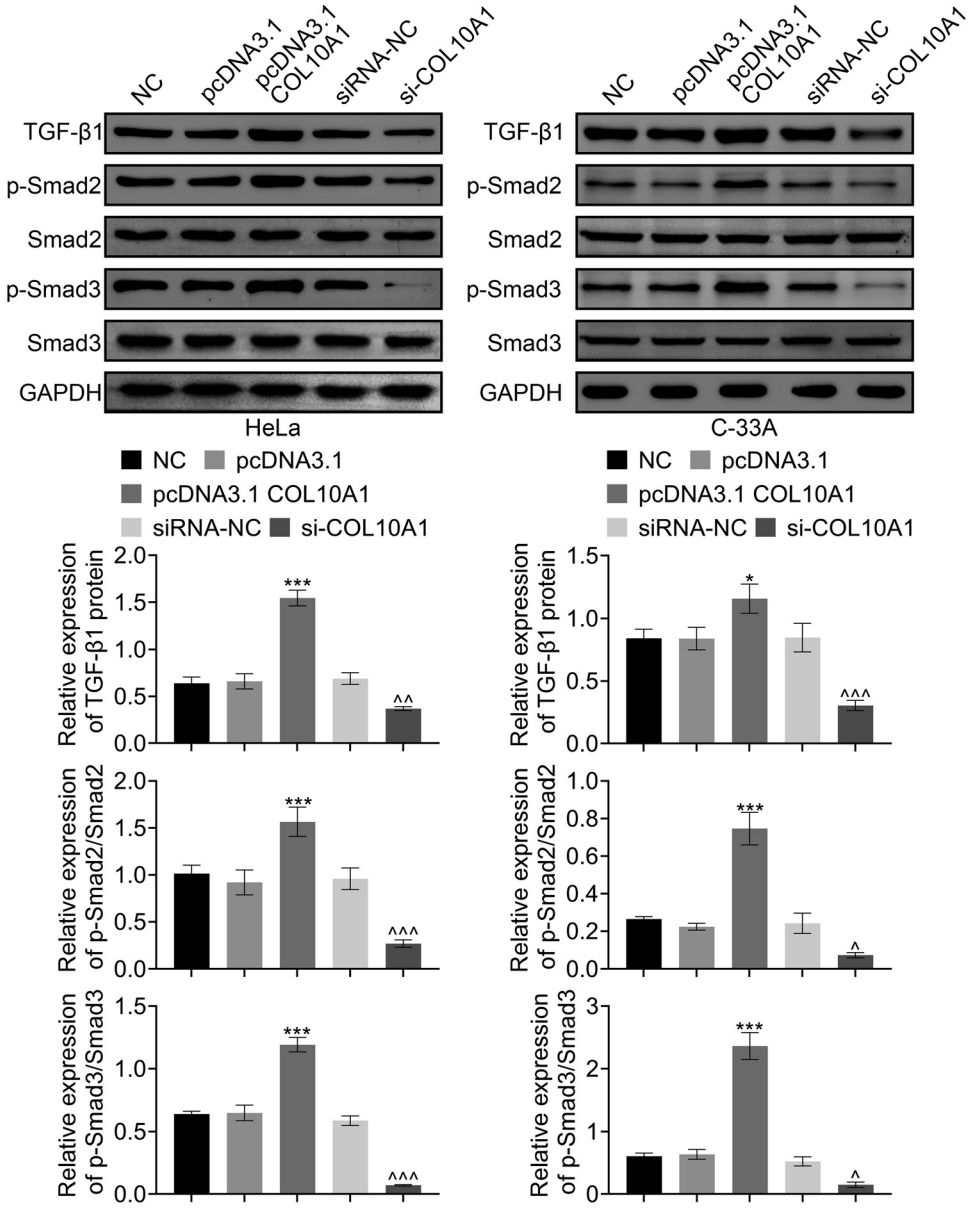

Fig. 5. COL10A1 contributed to activation of TGF-β/Smad signaling in cervical cancer Over-expression of COL10A1 enhanced protein expression of TGF-β1, phosphorylation of Smad2 and Smad3 in HeLa and C-33A, whereas knockdown of COL10A1 reduced TGF-β1, phosphorylation of Smad2 and Smad3. $n = 3$. *** vs. pcDNA3.1, $P < 0.001$. ^^, ^^^ vs. siRNA-NC, $P < 0.01$, $P < 0.001$

was inhibited by transfection with si-COL10A1 with reduced number of invasive cells (Fig. 3B). These results suggested the anti-invasive effect of COL10A1 silencing on CC.

COL10A1 contributed to EMT of CC. Protein expression of E-cadherin in HeLa and C-33A cells was reduced, whereas that of N-cadherin and Vimentin was enhanced by over-expression of COL10A1 (Fig. 4). However, transfection with si-COL10A1 up-regulated E-cadherin protein, and down-regulated N-cadherin and Vimentin (Fig. 4) to inhibit EMT of CC.

COL10A1 contributed to activation of TGF-β/Smad signaling in CC

Transfection with pcDNA3.1-COL10A1 enhanced protein expression of TGF-β1 in HeLa and C-33A cells (Fig. 5), whereas transfection with si-COL10A1 reduced the protein expression (Fig. 5). Protein levels of Smad2 and Smad3 were not affected by transfection with pcDNA3.1-COL10A1 or si-COL10A1 in HeLa and C-33A (Fig. 5). The phosphorylation of Smad2 and Smad3 was enhanced by over-expression of COL10A1 (Fig. 5), and decreased by silencing of COL10A1 (Fig. 5). These results indicated that inhibition of COL10A1 inhibited the activation of TGF-β/Smad signaling in CC.

DISCUSSION

Extracellular matrix is an abundant component in tumor microenvironment that modulates tissue tension homeostasis and tumor cell behaviors [20]. Collagens, the major components of the extracellular matrix are regarded as a scaffold of tumor microenvironment through promotion of angiogenesis, tumor infiltration, and metastasis [20]. Aberrant expression of collagens was found in CC [21], and COL1A1 regulated radiation-induced cell apoptosis in CC [22]. This study found the oncogenic role of COL10A1 in CC through promoting cell proliferation, metastasis and EMT.

Firstly, data based on the TCGA database showed that COL10A1 was elevated in CC tissues. Moreover, CC cells also expressed higher levels of COL10A1 than did cervical epithelial cells. Necula et al. have shown that higher expression of COL10A1 was associated with advanced tumor stage of gastric cancer, and COL10A1 might function as a diagnostic and prognostic biomarker for the tumor [23]. Therefore, the relation between COL10A1 and the clinical-pathological characteristics of patients with CC should be evaluated to identify the prognostic or diagnostic roles of COL10A1 in CC.

Liang et al. have shown that COL10A1 contributed to the malignant progression of lung adenocarcinoma through promoting of cell proliferation and metastasis [24]. Our results showed that silencing of COL10A1 reduced cell proliferation of CC, and suppressed migration and invasion. Xu et al. showed that epithelial cells underwent EMT and in this process, they lost polarity, specialized cell-cell contacts, and acquired migratory behavior, which contributed to cell metastasis of tumors [25]. Blockade of EMT was considered to be a potential strategy for CC [26]. COL10A1 has been shown to down-regulate E-cadherin, and up-regulate N-cadherin, Slug and Snail proteins to promote the EMT of colorectal cancer [9]. In our study, E-cadherin was enhanced, whereas N-cadherin and Vimentin were reduced by silencing of COL10A1 in CC cells, suggesting that knockdown of COL10A1 suppressed EMT of CC to inhibit the tumor metastasis.

Emerging evidence has shown that TGF-β1 induced EMT through phosphorylation of Smad2 and Smad3 [27]. Inhibition of TGF-β/Smad2/3 signaling contributed to the suppression

of epithelial→mesenchymal transition in ovarian cancer [28]. The phosphorylation level of Smad2 in gastric cancer cells was decreased by transfection with si-COL10A1, and silencing of COL10A1 inhibited EMT, as well as the migration and invasion abilities of gastric cancer cells [19]. Results in this study demonstrated that silencing of COL10A1 reduced TGF-β1 expression, and down-regulated phosphorylation of Smad2 and Smad3 in CC, indicating that COL10A1 contributed to EMT of CC through activation of TGF-β/Smad2/3 signaling.

In summary, COL10A1 was elevated in CC, and knockdown of COL10A1 suppressed cell proliferation, metastasis and EMT of CC through inactivation of TGF-β/Smad2/3 signaling. However, the effect of COL10A1 on cell apoptosis of CC, and the *in vivo* effects of COL10A1 on tumor growth of CC should be investigated in further research.

Competing interests: The authors state that there are no conflicts of interest to disclose.

REFERENCES

1. Duan J-Q, Huang H-G, Shui C-L, Jiang W, Luo X. The cardioprotective effect of dexmedetomidine on elderly patients with cervical cancer in the Trendelenburg position. Signa Vitae 2021; 17(5): 117–21. https://doi.org/10.22514/sv.2021.007.
2. Vu M, Yu J, Awolude OA, Chuang L. Cervical cancer worldwide. Curr Probl Cancer 2018; 42(5): 457–65. https://doi.org/10.1016/j.currproblcancer.2018.06.003.
3. Elshami M, Thalji M, Abukmail H, Al-Slaibi I, Alser M, Radaydeh A, et al. Knowledge of cervical cancer risk factors among Palestinian women: a national cross-sectional study. BMC Womens Health 2021; 21(1): 385. https://doi.org/10.1186/s12905-021-01510-2.
4. Small, Jr W, Bacon MA, Bajaj A, Chuang LT, Fisher BJ, Harkenrider MM, et al. Cervical cancer: a global health crisis. Cancer 2017; 123(13): 2404–12. https://doi.org/10.1002/cncr.30667.
5. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61(2): 69–90. https://doi.org/10.3322/caac.20107.
6. Zhang X, Liang H, Liu W, Li X, Zhang W, Shang X. A novel sequence variant in COL10A1 causing spondylometaphyseal dysplasia accompanied with coxa valga: a case report. Medicine (Baltimore) 2019; 98(30): e16485. https://doi.org/10.1097/MD.0000000000016485.
7. Chen S, Wei Y, Liu H, Gong Y, Zhou Y, Yang H, et al. Analysis of Collagen type X alpha 1 (COL10A1) expression and prognostic significance in gastric cancer based on bioinformatics. Bioengineered 2021; 12(1): 127–37. https://doi.org/10.1080/21655979.2020.1864912.
8. Zhang M, Chen H, Wang M, Bai F, Wu K. Bioinformatics analysis of prognostic significance of COL10A1 in breast cancer. Biosci Rep 2020; 40(2):BSR20193286. https://doi.org/10.1042/BSR20193286.
9. Huang H, Li T, Ye G, Zhao L, Zhang Z, Mo D, et al. High expression of COL10A1 is associated with poor prognosis in colorectal cancer. Onco Targets Ther 2018; 11: 1571–81. https://doi.org/10.2147/OTT.S160196.
10. Zhou L, Li SH, Wu Y, Xin L. Establishment of a prognostic model of four genes in gastric cancer based on multiple data sets. Cancer Med 2021; 10(10): 3309–22. https://doi.org/10.1002/cam4.3654.
11. Solé X, Crous-Bou M, Cordero D, Olivares D, Guinó E, Sanz-Pamplona R, et al. Discovery and validation of new potential biomarkers for early detection of colon cancer. PLoS One 2014; 9(9): e106748. https://doi.org/10.1371/journal.pone.0106748.
12. Song Y, Wang X, Wang F, Peng X, Li P, Liu S, et al. Identification of four genes and biological characteristics of esophageal squamous cell carcinoma by integrated bioinformatics analysis. Cancer Cell Int 2021; 21(1): 123. https://doi.org/10.1186/s12935-021-01814-1.

13. Li Y, Wang X, Shi L, Xu J, Sun B. Predictions for high *COL1A1* and *COL10A1* expression resulting in a poor prognosis in esophageal squamous cell carcinoma by bioinformatics analyses. Transl Cancer Res 2020; 9(1): 85–94. https://doi.org/10.21037/tcr.2019.11.11.
14. Li H-H, Wang J-D, Wang W, Wang H-F, Lv J-Q. Effect of miR-26a-5p on gastric cancer cell proliferation, migration and invasion by targeting COL10A1. Eur Rev Med Pharmacol Sci 2020; 24(3): 1186–94. https://doi.org/10.26355/eurrev_202002_20170.
15. Liu S, Chen S, Zeng J. TGF-β signaling: a complex role in tumorigenesis (Review). Mol Med Rep 2018; 17(1): 699–704. https://doi.org/10.3892/mmr.2017.7970.
16. Karunagaran D, Jinesh G. TGF-β, Smads and cervical cancer. In: Jakowlew SB, editor. Transforming growth factor-beta in cancer therapy, volume II: cancer treatment and therapy. Totowa, NJ: Humana Press Inc.; 2008. pp. 33–49.
17. Deng M, Cai X, Long L, Xie L, Ma H, Zhou Y, et al. CD36 promotes the epithelial-mesenchymal transition and metastasis in cervical cancer by interacting with TGF-β. J Transl Med 2019; 17(1): 352. https://doi.org/10.1186/s12967-019-2098-6.
18. Nishizuka M, Komada R, Imagawa M. Knockdown of RhoE expression enhances TGF-β-induced EMT (epithelial-to-mesenchymal transition) in cervical cancer HeLa cells. Int J Mol Sci 2019; 20(19): 4697. https://doi.org/10.3390/ijms20194697.
19. Li T, Huang H, Shi G, Zhao L, Li T, Zhang Z, et al. TGF-β1-SOX9 axis-inducible COL10A1 promotes invasion and metastasis in gastric cancer via epithelial-to-mesenchymal transition. Cell Death Dis 2018; 9(9): 849. https://doi.org/10.1038/s41419-018-0877-2.
20. Fang M, Yuan J, Peng C, Li Y. Collagen as a double-edged sword in tumor progression. Tumour Biol 2014; 35(4): 2871–82. https://doi.org/10.1007/s13277-013-1511-7.
21. Thangavelu PU, Krenács T, Dray E, Duijf PHG. In epithelial cancers, aberrant *COL17A1* promoter methylation predicts its misexpression and increased invasion. Clin Epigenetics 2016; 8(1): 120. https://doi.org/10.1186/s13148-016-0290-6.
22. Liu S, Liao G, Li G. Regulatory effects of COL1A1 on apoptosis induced by radiation in cervical cancer cells. Cancer Cell Int 2017; 17: 73. https://doi.org/10.1186/s12935-017-0443-5.
23. Necula L, Matei L, Dragu D, Pitica I, Neagu AI, Bleotu C, et al. High plasma levels of COL10A1 are associated with advanced tumor stage in gastric cancer patients. World J Gastroenterol 2020; 26(22): 3024–33. https://doi.org/10.3748/wjg.v26.i22.3024.
24. Liang Y, Xia W, Zhang T, Chen B, Wang H, Song X, et al. Upregulated collagen COL10A1 remodels the extracellular matrix and promotes malignant progression in lung adenocarcinoma. Front Oncol 2020; 10: 573534. https://doi.org/10.3389/fonc.2020.573534.
25. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. Cell Res 2009; 19(2): 156–72. https://doi.org/10.1038/cr.2009.5.
26. Lee M-Y, Chou C-Y, Tang M-J, Shen M-R. Epithelial-mesenchymal transition in cervical cancer: correlation with tumor progression, epidermal growth factor receptor overexpression, and snail up-regulation. Clin Cancer Res 2008; 14(15): 4743. https://doi.org/10.1158/1078-0432.CCR-08-0234.
27. Yu C, Liu Y, Huang D, Dai Y, Cai G, Sun J, et al. TGF-β1 mediates epithelial to mesenchymal transition via the TGF-β/Smad pathway in squamous cell carcinoma of the head and neck. Oncol Rep 2011; 25(6): 1581–7. https://doi.org/10.3892/or.2011.1251.
28. An Q, Liu T, Wang M-Y, Yang Y-J, Zhang Z-D, Liu Z-J, et al. KRT7 promotes epithelial-mesenchymal transition in ovarian cancer via the TGF-β/Smad2/3 signaling pathway. Oncol Rep 2021; 45(2): 481–92. https://doi.org/10.3892/or.2020.7886.