Syndecan-1 suppresses epithelial-mesenchymal transition and migration in human oral cancer cells

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Abstract. Epithelial-mesenchymal transition (EMT) is one of the major processes that contribute to the occurrence of cancer metastasis. EMT has been associated with the development of oral cancer. Syndecan-1 (SDC1) is a key cell-surface adhesion molecule and its expression level inversely correlates with tumor differentiation and prognosis. In the present study, we aimed to determine the role of SDC1 in oral cancer progression and investigate the molecular mechanisms through which SDC1 regulates the EMT and invasiveness of oral cancer cells. We demonstrated that basal SDC1 expression levels were lower in four oral cancer cell lines (KB, Tca8113, ACC2 and CAL-27), than in normal human periodontal ligament fibroblasts. Ectopic overexpression of SDC1 resulted in morphological transformation, decreased expression of EMT-associated markers, as well as decreased migration, invasiveness and proliferation of oral cancer cells. In contrast, downregulation of the expression of SDC1 caused the opposite results. Furthermore, the knockdown of endogenous SDC1 activated the extracellular signal-regulated kinase (ERK) cascade, upregulated the expression of Snail and inhibited the expression of E-cadherin. In conclusion, our findings revealed that SDC1 suppressed EMT via the modulation of the ERK signaling pathway that, in turn, negatively affected the invasiveness of human oral cancer cells. Our results provided useful evidence about the potential use of SDC1 as a molecular target for therapeutic interventions in human oral cancer.

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Introduction

Oral cancer is the fifth most common type of cancer in the world (1). Despite modern treatment modalities, improvement in survival rates over the past decade has been minimal. The development of local recurrence, the formation of second primary tumors and metastasis are the principal reasons of this poor outcome (2). Therefore, recent studies in this area have focused on potential biomarkers for the early diagnosis of oral cancer and the continuous monitoring and prediction of prognosis of patients with oral cancer (3,4).

Epithelial-mesenchymal transition (EMT) is known to be one of the key mechanisms leading to cancer metastasis (5). It is a process that causes epithelial cells to lose their polarity and intercellular contacts and obtain transport properties of mesenchymal cells. In addition, the expression of mesenchymal markers is increased and changes in the cytoskeleton occur. These changes render epithelial cells more mobile and invasive as they acquire the appearance of mesenchymal cells (6,7). Many signaling cascades, such as the transforming growth factor-β (TGF-β) (8), wingless (Wnt) (9) and Notch pathway (10) can induce EMT. It is known that EMT can also be regulated by several development factors, such as Snail1, Snail2, zinc finger E-box binding homeobox 1 (ZEB1) and forkhead box protein C2 (FOXC2) (11-13). Although the correlation between EMT and cancer progression has been established, the underlying molecular mechanism of this relationship remains largely unclear.

Syndecan-1 (SDC1) is one of the key cell-surface adhesion molecules that regulate cell and extracellular matrix adhesion and cell migration. It also regulates tumor-cell survival, proliferation, angiogenesis and metastasis and ultimately affects tumorigenesis (14,15). Recent studies revealed that in a number of cancers, such as head and neck, ovarian, breast and colorectal carcinomas, the expression of SDC1 is dysregulated (16-19). Despite a large number of published studies, the precise mechanisms that explain the role of SDC1 in these pathologies are still not fully elucidated.

In the present study, we observed that the expression of SDC1 was frequently downregulated in oral cancer cell lines. Exogenous overexpression of SDC1 led to the morphological transformation of cells, attenuated the expression of EMT markers and inhibited proliferation, migration and invasion of oral cancer cells. Furthermore, the knockdown of endogenous

SDC1 activated the extracellular signal-regulated kinase (ERK) cascade, upregulated the expression of Snail and inhibited the expression of E-cadherin. These regulatory properties revealed novel mechanisms of the involvement of SDC1 in EMT and the invasive phenotype of human oral cancer cells. Our data demonstrated that SDC1 is a key negative regulator of EMT and a potential target for therapeutic intervention in oral cancer.

Materials and methods

Cell lines. The KB, Tca8113, ACC2 and CAL27 cell lines were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Human periodontal ligament (hPDL) fibroblasts were purchased from ScienCell Research Laboratories (Carlsbad, CA, USA). KB cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Rockford, IL, USA) containing 10% fetal bovine serum (FBS; Kang Yuan Biological Technology Co., Ltd., Tianjin, China). Tca8113, ACC2 and CAL27 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Grand Island, NY, USA) supplemented with 10% FBS (Kang Yuan Biological Technology). The hPDLF cells were cultured in DMEM/F12 (Thermo Fisher Scientific) supplemented with 10% FBS (Biological Industries, Cromwell, CT, USA). The cell cultures were maintained at 37˚C in a humidified atmosphere of 95% air and 5% CO_2.

Quantitative PCR (qPCR). Total RNA was reverse transcribed using the RT system supplied by Promega (cat. no. A1702; Madison, WI, USA) and qPCR was performed on a Mastercycler supplied by Eppendorf (Hamburg, Germany). The PCR primer sequences were as follows: hSDC1 sense, AGGACGAAGGCAGCTACTCCT and antisense, TTTGGT GGGCTTCTGGTAGG; β-actin sense, GAGCACAGAGCC TCGCCTTT and antisense, ATCCTTCTGACCCATGCCCA; E-cadherin sense, GACAACAAGCCCGAATT and antisense, GGAAACTCTCTCGGTCCA; vimentin sense, GAGAAC TTTGCCGTTGAAGC and antisense, GCTTCCTGTAGG TGGCAATC; Snail sense, GCAAATACTGCAACAAGG and antisense, GCACTGGTACTTCTTGACA; Slug sense, AGATGCATATTCGGACCCAC and antisense, CCTCAT GTTTGTGCAGGAGA; Twist sense GGAGTCCGCAGTCTT ACGAG and antisense, TCTGGAGGACCTGGTAGAGG.

Plasmids and stable cell lines. The pTT5-hSDC1 plasmid (cat. no. 52326; Addgene, Inc., Cambridge, MA, USA) was donated by Professor Gordon Laurie (University of Virginia). Oral cancer cells were transfected with pTT5-hSDC1 or control plasmids, using Lipofectamine™ LTX with PLUS™ reagent (cat. no. 15338100; Invitrogen; Thermo Fisher Scientific, Carlsbad, CA, USA).

Short hairpin RNA (shRNA) plasmids were obtained from Shanghai GeneChem (Shanghai, China). Lentiviruses were produced by co-transfecting 293T cells with one of the shRNA expression plasmids and the packaging plasmids (psPAX2 and pMD2.G). The supernatants were collected 48 h after transfection, filtered through 0.45-mm filters (cat. no. SLGV033RB; EMD Millipore, Temecula, CA, USA) and concentrated using 100 kDa MWCO Amicon Ultra centrifugal filters (cat. no. Z648043; EMD Millipore). Stable cells infected with shSDC1 and shCtrl (control) were selected on 100 μg/ml hygromycin (Sigma-Aldrich, St. Louis, MO, USA; cat. no. H0654) for about two weeks, as previously described (10).

Western blot analysis. The experiments were performed as previously described (10). The protein lysates were resolved by SDS-PAGE, transferred to PVDF membranes (cat. no. ISEQ00010; EMD Millipore), detected with primary antibody overnight at 4˚C and then incubated with HRP-conjugated secondary antibodies for 90 min at 25˚C. Western blots were visualized using ECL reagents (cat. nos. RPN2232 and RPN2236; GE Healthcare, Pittsburgh, PA, USA). The following antibodies were used: Rabbit monoclonal anti-E-cadherin antibody (cat. no. 4065) and Rabbit polyclonal anti-N-cadherin antibody (cat. no. 4061) (both 1:1,000 dilution; Cell Signaling Technology, Inc., Beverly, MA, USA); rabbit monoclonal anti-SDC1 antibody (cat. no. ab128936) and rabbit polyclonal anti-Snail antibody (cat. no. ab63371) (both 1:2,000 dilution; Abcam, Hong Kong, China); mouse monoclonal anti-vimentin antibody (cat. no. 550513), mouse monoclonal anti-β-catenin antibody (cat. no. 610153) (both 1:1,000 dilution; BD Biosciences, San Jose, CA, USA); mouse monoclonal anti-β-actin antibody (1:10,000, dilution; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany); HRP-conjugated secondary antibodies (1:1,000 dilution; cat. no. SC-2048; ZSGB-BIO, Beijing, China).

Hoechst 33342 staining. The cells ($1x10^4$/well) were seeded in 96-well plates, stained with Hoechst 33342 (cat. no. B2261; Sigma-Aldrich) and observed by fluorescence microscopy. The number of apoptotic cells in five random fields of view was counted and apoptotic characteristics were recorded.

Colony formation assay. The cells ($1x10^3$/well) were seeded in 6-well plates and selected with 0.1 mg/l puromycin for 14 days. The colonies were stained with crystal violet (cat. no. C3886; Sigma-Aldrich).

Proliferation assay. 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assays were carried out to detect cell proliferation. Cells were plated on 96-well plates at a density of $1x10^4$ cells/well. The absorbance of each well was measured at 492 nm using the Take3 microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). The survival percentage (%) was calculated relative to that observed in the control cells.

In vitro migration and invasion assay. *In vitro* migration and invasion assays were performed using 24-well Transwell plates with polycarbonate filters (Costar; Corning Life Sciences, Cambridge, MA, USA), as previously described (20). For the migration assay, $2.5x10^4$ cells were added to the upper insert. For the invasion assay, $5x10^4$ cells were seeded into the upper insert coated with Matrigel (BD Biosciences). The cells were observed with an optical microscope connected to a camera and the number of migrated cells was assessed by randomly capturing ten images from each membrane.

Immunofluorescence. Cells ($1x10^5$/well) were grown on glass coverslips in a 6-well plate, washed three times with phosphate-buffered saline (PBS), then fixed in 4% formaldehyde and permeabilized with 0.1% Triton X-100 in PBS for 5 min. The cells were blocked with 2% bovine serum albumin (BSA) in PBS for 30 min at 20-25˚C. Coverslips were incubated with the respective primary antibodies at 1:100 dilution for 1 h and then washed with PBS and incubated for 1 h with tetramethylrhodamine (TRITC)-conjugated secondary antibodies at 1:50 dilution (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China). The cells were further washed in PBS and mounted with Vectashield mounting medium (Vector Laboratories, Inc., Burlingame, CA, USA) containing 4',6-diamidino-2-phenylindole (DAPI) and were analyzed using fluorescence microscopy. Images were captured under a Nikon (Tokyo, Japan) microscope with a fluorescein isothiocyanate filter.

Statistical analysis. Statistical significance of differences between experimental group values is assessed using Student's t-test. The levels of statistical significance are set as follows, $P<0.05$ and $P<0.01$. Error bars denote the standard deviation (SD).

Results

SDC1 expression is downregulated in oral cancer cells. We first evaluated SDC1 expression in four human oral carcinoma cell lines, KB (oral epidermoid carcinoma cells), Tca8113 (tongue squamous cells), ACC2 (adenoid cystic carcinoma) and CAL27 (tongue squamous cells), by qPCR and western blotting. As displayed in Fig. 1A and B, all four oral cancer cell lines exhibited lower levels of SDC1 mRNA and protein, when compared with hPDL fibroblasts.

SDC1 induces apoptosis and inhibits growth and colony formation in oral cancer cells. To study the functional significance of low SDC1 expression in oral cancer, we tested the effect of SDC1 on proliferation and apoptosis in oral cancer cells. Firstly, we confirmed the expression levels of SDC1 in Tca8113-SDC1 and ACC2-SDC1 cells, both overexpressing SDC1, by immunoblotting (Fig. 1C). Subsequently, we carried out MTT assays to study the effect of SDC1 on the proliferation of oral cancer cells. We found that the overexpression of SDC1 significantly inhibited cell proliferation, particularly 48 and 72 h after transfection (Fig. 1D and E). To further assess the role of SDC1 in oral cancer cells, we knocked down endogenous SDC1 by shRNA interference in Tca8113 and ACC2 cells (Fig. 1F). MTT assays showed that SDC1 knockdown promoted the growth of Tca8113 and ACC2 cells (Fig. 1G and H). These results indicated that SDC1 can inhibit cell growth in oral cancer cells.

Furthermore, colony formation assays confirmed that overexpression of SDC1 inhibited cell proliferation while interfering with endogenous SDC1 expression significantly increased the number of cell colonies (Fig. 1I). These results indicated that SDC1 expression inhibited colony formation in oral cancer cells. Hoechst 33342 staining revealed that overexpression of SDC1 promoted apoptosis in oral cancer cells (Fig. 1J). Fluorescence microscopy revealed that the nuclei of the control cells were round and stained evenly. At the same time, SDC1-overexpressing cells exhibited typical morphological features of apoptotic cells, such as cell shrinkage, chromatin condensation and apoptotic bodies as shown by the arrows. However, when we knocked down endogenous SDC1 by shRNA interference, we found that the nuclei of both SDC1-knockdown and control group cells had regular morphology and nuclear membrane integrity and did not reveal significant apoptosis.

SDC1 suppresses migration and invasion in oral cancer cells. Subsequently, we explored the role of SDC1 in cell migration and invasion. As displayed in Fig. 2A, the wound healing rate of Tca8113-SDC1 cells was only half of that observed in the control group 24 h after the generation of the wound. Transwell migration and invasion assays revealed that the abilities of Tca8113-SDC1 cells to migrate and invade were much lower than those of the control cells (Fig. 2B and C). Opposite results were obtained upon SDC1 RNA interference in oral cancer cells: As displayed in Fig. 2D, the wound healing rate of SDC1-knockdown cells was higher than that of the control group, indicating that SDC1-knockdown cells had greater migratory ability. In addition, SDC1 knockdown in ACC2 cells enhanced the migratory and invasive properties of the cells (Fig. 2E and F).

SDC1 regulates EMT. We intended to clarify the biological role of SDC1 imbalance in the development of oral cancer. We first examined whether the overexpression of SDC1 inhibited EMT in Tca8113 human tongue squamous cell carcinoma cells, which express low levels of SDC1. Tca8113 cells expressing a control vector displayed a spindle-like, fibroblastic phenotype. In contrast, Tca8113-SDC1 cells, overexpressing SDC1, demonstrated significantly polarized epithelial cell morphology with strong intercellular cell adhesion (Fig. 3A, upper panel). In addition to morphological changes, overexpression of SDC1 caused changes in the expression of epithelial and mesenchymal markers. Specifically, both at the mRNA and protein levels, the expression of E-cadherin, an epithelial marker, in Tca8113-SDC1 cells was significantly upregulated, whereas the expression levels of N-cadherin and vimentin, mesenchymal markers, were greatly reduced (Fig. 3B and C). The observed change was further validated by the examination of the subcellular localization of proteins by immunofluorescent staining. As expected, immunofluorescence microscopy revealed high E-cadherin staining intensity in the cell membrane of Tca8113-SDC1 cells, whereas only weak membrane staining was noted in the control cells. Staining for vimentin exhibited an opposite pattern (Fig. 3D).

Subsequently, we examined whether the inhibition of endogenous SDC1 expression induced EMT progression in oral cancer cells. ACC2 cells expressing shSDC1 displayed spindle-like, fibroblastic morphology (Fig. 3A, bottom panel). Additionally, using qPCR and western blotting, we demonstrated that after SDC1 knockdown in ACC2 cells, the expression of mesenchymal markers such as N-cadherin and vimentin was higher, whereas the expression of the epithelial markers E-cadherin and occludin (data not shown) was lower than that in control cells (Fig. 3B and C). We obtained similar

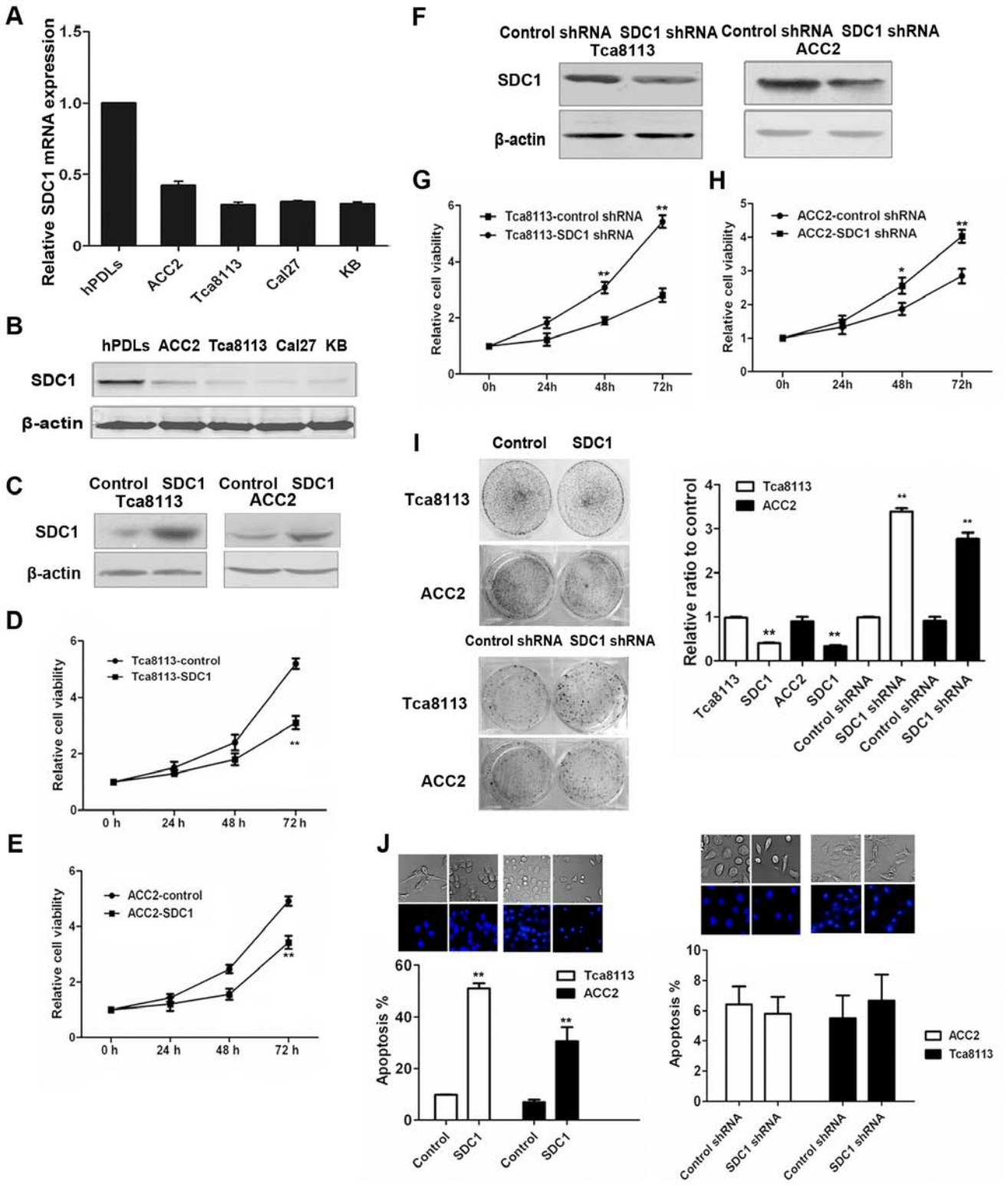

Figure 1. SDC1 inhibits proliferation in oral cancer cells. (A) qPCR and (B) western blot analysis of the expression of SDC1 in human periodontal ligament (hPDL) fibroblasts and oral cancer cell lines. (C) SDC1 expression in Tca8113 and ACC2 cells was assessed by western blotting. (D and E) Effect of SDC1 overexpression on cell proliferation. MTT assays were performed to detect cell proliferation at 0, 24, 48 and 72 h after transfection. (F) Immunoblots of the interference efficiency of SDC1 in oral cancer cells. (G and H) The effect of SDC1 silencing on cell proliferation. (I) The role of SDC1 on colony formation. (J) The effect of SDC1 on apoptosis. The cells were stained with Hoechst 33342 and apoptotic cells were observed by fluorescence microscopy and counted. Error bars, mean ± SD. *P<0.05; **P<0.01.

results in immunofluorescence experiments. We observed that, upon SDC1 knockdown, E-cadherin staining intensity was attenuated, whereas staining for vimentin was significantly enhanced (Fig. 3D). These results indicated that inhibition of SDC1 expression in oral cancer cells increased cell migration and invasion and induced EMT.

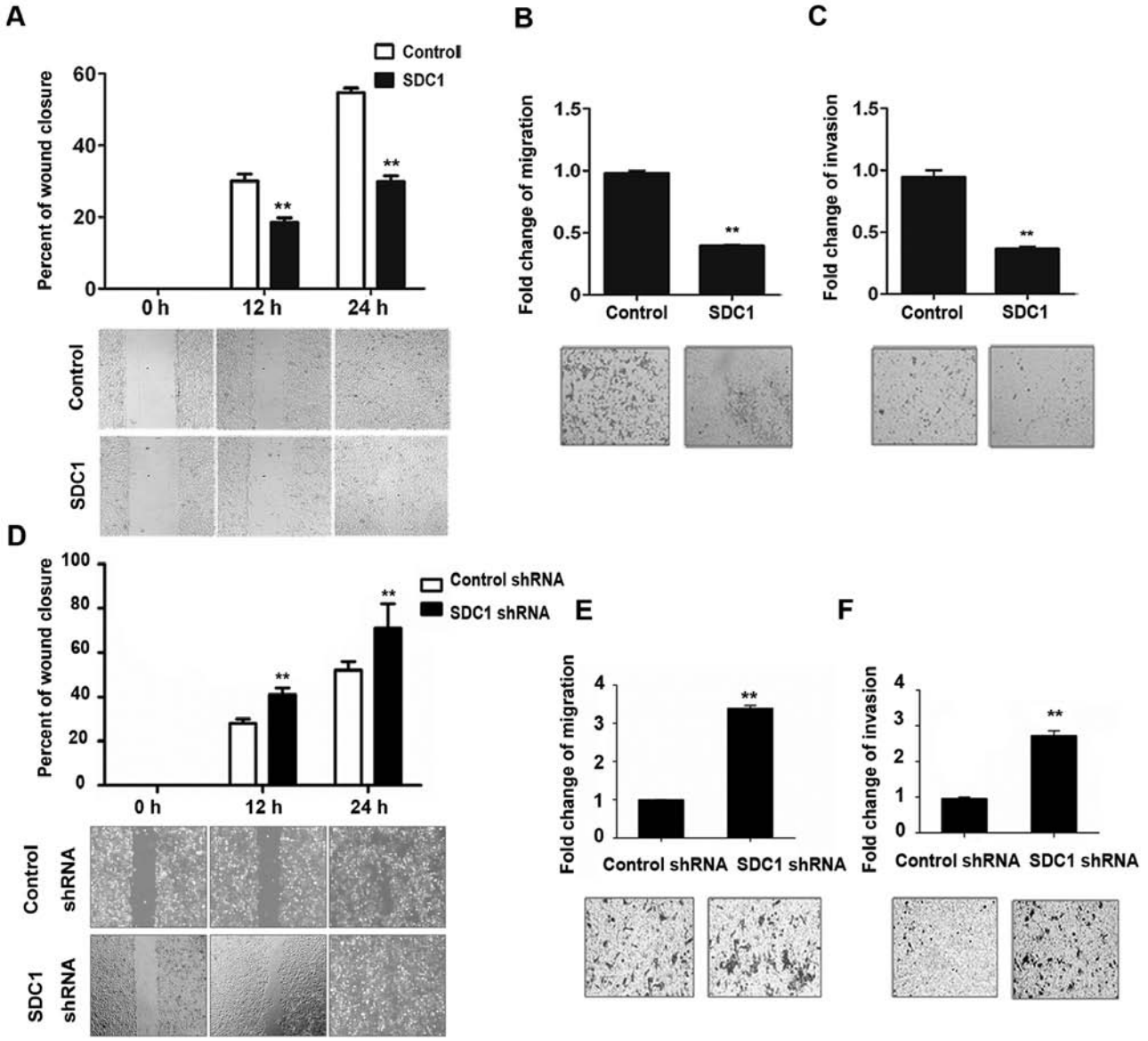

Figure 2. Effect of SDC1 on cell migration and invasion. (A) The effect of SDC1 overexpression on cell migration in wound healing assays. The cells were seeded in a 6-well plate and a scratch was made. Images were captured at 0, 12 and 24 h after the scratches. The ImageJ software was used to calculate the percentage of wound closures. Scale bars, 200 μm. (B and C) Migration and invasion assays in Tca8113-SDC1 and control cells, respectively. The cells were starved for 18 h before cell migration and invasion assays were performed using Matrigel Transwell filters. The migrated and invaded cells were stained and counted. Representative images from each group are displayed. (D) Wound-healing assay to assess the effect of SDC1 on cell mobility in ACC2-SDC1 shRNA and control cells. Scale bars, 200 μm. (E and F) Migration and invasion assays inACC2-SDC1 shRNA and control cells, respectively. Error bars, mean ± SD. *P<0.05; **P<0.01.

SDC1 regulates EMT through the ERK-Snail signaling. We found that the overexpression of SDC1 increased the levels of E-cadherin mRNA expression (Fig. 4A). Snail, Slug, Twist and ZEB are transcription factors that control the expression of E-cadherin in EMT (7,12,13). Therefore, we examined whether the expression of these factors is affected by SDC1 levels in oral cancer cells. qPCR analysis revealed that the expression levels of ZEB1, ZEB2, inhibitor of DNA binding (ID)2, Slug and Twist were similar in Tca8113-SDC1 and control cells (Fig. 4B), whereas Snail expression level was significantly lower in Tca8113-SDC1 cells. Western blot analysis confirmed these results (Fig. 4C, third line).

It is known that the activation of the ERK signaling pathway induces Snail expression (21,22). Therefore, we examined whether the ERK1 signaling pathway is involved in the regulation of Snail expression mediated by SDC1. Western blot analysis revealed that the overexpression of SDC1 inhibited the phosphorylation of ERK and suppressed Snail expression (Fig. 4C, left panel). In contrast, the levels of phosphorylated ERK and Snail in shSDC1-ACC2 cells were significantly higher than those in the control cells. These results indicate that the ERK signaling pathway may function downstream of SDC1 (Fig. 4C, right panel).

Collectively, our data indicated that downregulation of endogenous SDC1 activated the ERK cascade, upregulated Snail expression and inhibited E-cadherin expression. These changes may enhance the understanding of the relationship between EMT and human oral cancer invasion.

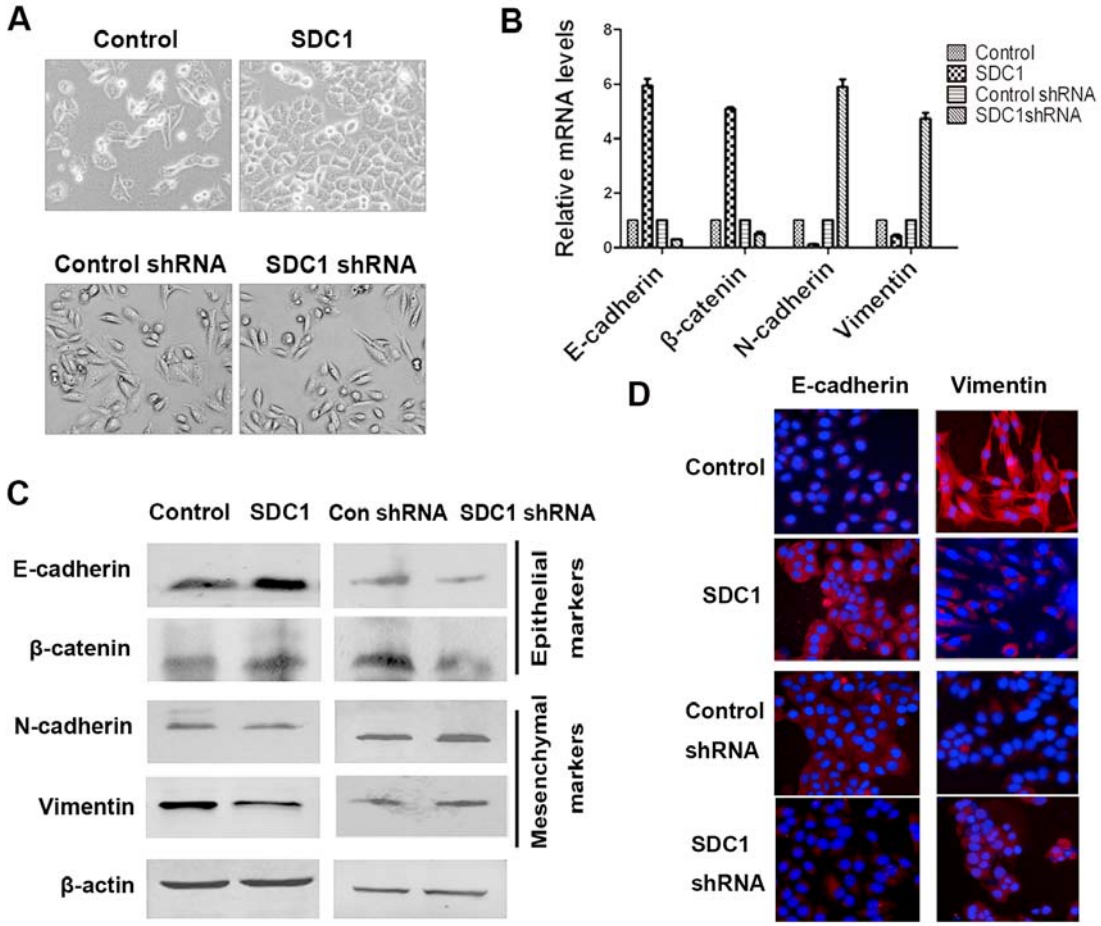

Figure 3. SDC1 regulates EMT. (A) The morphology of cells was observed by phase-contrast microscopy. Scale bar, 100 μm. (B) The mRNA expression levels of EMT markers were assessed by qPCR. (C) Immunoblotting analysis of EMT markers. (D) Immunofluorescence staining for EMT makers. Scale bar, 100 μm.

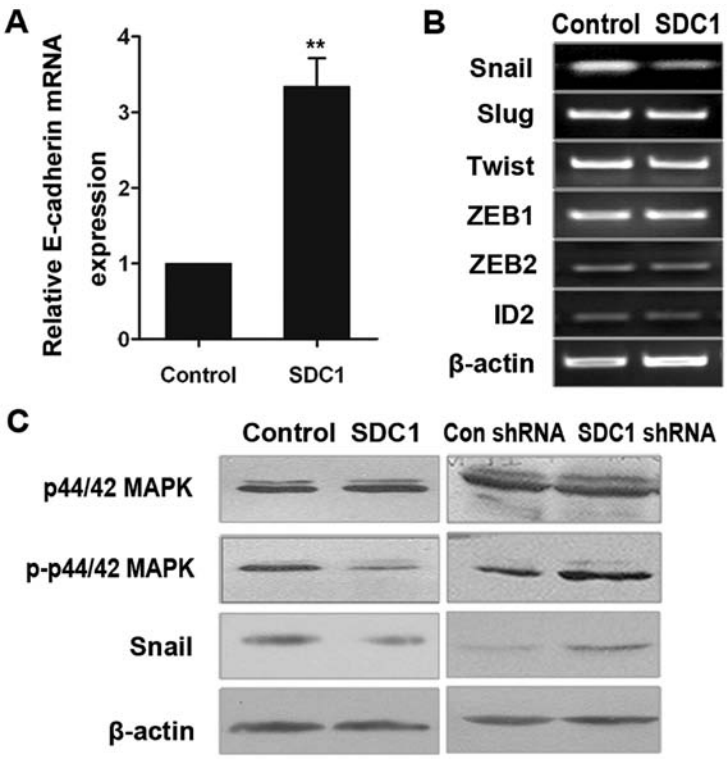

Figure 4. Molecular EMT events regulated by SDC1. (A) SDC1 increased E-cadherin mRNA expression in oral cancer cells. Error bars, mean ± SD. *P<0.05; **P<0.01. (B) qPCR analyses of EMT inducers in Tca8113-SDC1 and control Tca8113 cells. (C) Western blot analysis of the ERK activation.

Discussion

It has been previously demonstrated that the levels of SDC1 expression in cancer cells inversely correlate with the extent of tumor differentiation and prognosis. In the present study, we investigated the role of SDC1 in the progression of oral cancer and found that SDC1 inhibited EMT and thereby affected the infiltration of tumor cells in the oral cavity tissues.

SDC1 basal expression level and cell location are significant for understanding the occurrence, development, diffusion and infiltration of tumors. Although SDC1 has multiple functions, its mechanism of action and precise role in oral cancer has so far remained unclear. An important finding of the present study was that the oral cancer cell lines KB, Tca8113, ACC2 and CAL-27 exhibited low levels of SDC1 expression. As displayed in Fig. 1, the expression of SDC1 in all four oral cancer cell lines was significantly lower than that in functionally similar hPDL fibroblasts at both mRNA and protein levels. Ectopic overexpression of SDC1 led to the suppression of migration, invasion and proliferation of oral cancer cells. Our results were consistent with the observations of Kurokawa *et al* (23), who reported that decreased expression of SDC1 could be an effective marker of the histological grade of malignancy before deep tumor invasion of oral squamous cell carcinoma. Similarly, Muramatsu *et al* (24) examined the expression of SDC1 in seven different human oral cancer cell lines (HSC2, HSC3, HSC4, Ca9-22, SAS, KB and BSC-OF) and found that SDC1 was involved in the growth and invasiveness of tumor cells (24). These studies indicated that manipulating SDC1 levels may be useful to inhibit the progression of oral cancer. However, the functions of SDC1 in patients and mouse models still need to be further investigated.

EMT has been demonstrated to promote tumor invasion and metastasis by conferring a mesenchymal cell phenotype to cancer cells. It has been previously demonstrated that EMT is important for oral cancer progression (25-27). In the present study, we observed that overexpression of SDC1 resulted in changes in cell morphology and attenuated the molecular manifestations of EMT. In contrast, suppression of endogenous SDC1 expression inhibited the expression of E-cadherin and promoted EMT progression in oral cancer cells. These findings revealed that SDC1 may be a key negative regulator of EMT. Consistent with our results, previous studies have reported the role of SDC1 in EMT. Leppa *et al* (28) found that SDC1 overexpression imparted epithelial-like morphology on tumorigenic mammary cell lines. Transfection of mammary cell lines with antisense RNA specific for E-cadherin suppressed SDC1 expression. Conversely, transfection with antisense SDC1 led to downregulation of the expression of E-cadherin. Simultaneous loss of SDC1 and E-cadherin expression was observed in the embryonic palate during EMT (29). Masola *et al* (30) reported that the interplay between heparanase and SDC1 is important fibroblast growth factor-2-induced EMT in renal tubular cells. Additionally, SDC1 expression pattern in prostate cancer indicated the involvement of this protein in EMT and tumor progression (31). The aforementioned data indicated that SDC1 expression levels negatively correlated with EMT progression and our results are in agreement with these findings.

Furthermore, the findings of the present study demonstrated that SDC1 knockdown activated the ERK signaling pathway, upregulated Snail expression and inhibited E-cadherin expression. These changes ultimately led to the occurrence of EMT in human oral cancer cells, thereby making tumor cells more aggressive. Several studies revealed that SDC1 and ERK/Snail signaling have a close relationship in tumor development process. Poblete *et al* (32) analyzed the expression of Snail, SDC1 and other EMT markers in a tissue microarray of prostate cancer samples and prostate cancer cell lines and, consistent with our results, demonstrated that increased Snail expression and low SDC1 levels were associated with high Gleason grade. Additionally, it is known that activation of the ERK signaling pathway can induce Snail expression in human breast and gastric cancer, and lung adenocarcinoma (21,22). In contrast, some previous studies have indicated that increased ERK activity enhanced SDC1 expression. Heidari-Hamedani *et al* (33) reported that ERK1/2 activity was enhanced six-fold upon SDC1 overexpression in malignant mesothelioma. Ju *et al* (34) found that SDC1/integrin interaction was essential in the activation of ERK I/II by insulin in osteoblast cells. These discrepancies may be due to distinct functions of SDC1 in different types of tumors. SDC1 may play an oncogenic function in breast cancer, lung cancer and glioma, whereas may be a tumor suppressor in prostate cancer and oral cancer. Further investigations to address these issues are required.

In conclusion, we demonstrated that SDC1 expression is downregulated in oral cancer cell lines. Overexpression of SDC1 reduced the expression of mesenchymal markers in oral cancer cells, increased the expression of epithelial markers and inhibited invasion, migration and proliferation of oral cancer cells. Knockdown of endogenous SDC1 led to morphological transformation of cells to mesenchymal phenotype, increased the expression levels of mesenchymal markers and enhanced cell migration, invasion and proliferation. Furthermore, knockdown of SDC1 in oral cancer cells activated the ERK signaling pathway, upregulated the expression of the EMT-inducing transcription factor Snail and inhibited the expression of E-cadherin. The results of the present study will help to elucidate the mechanism by which SDC1 affects EMT in tumor progression. In addition, the present study provided useful insights for the potential use of SDC1 as a molecular target for the treatment of oral cancer.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

TZ and CK conceived and designed the study. TQ participated in the concept of the study providing experimental ideas for the

mechanism research part. XW, CK, JH and XZ performed the experiments. TQ provided some financial support for the experiment. CK and XW wrote the paper. CK, TZ, TQ and XW reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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