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SOSTDC1 inhibits follicular thyroid cancer cell proliferation, migration, and EMT via suppressing PI3K/Akt and MAPK/Erk signaling pathways

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Abstract The sclerostin domain containing protein 1 (SOSTDC1) is a cell signaling regulator involved in cell physiology and pathology. SOSTDC1 is known to have a suppressive effect on certain kinds of cancer. However, the role of SOSTDC1 in follicular thyroid cancer (FTC) remains unknown. We aimed to investigate if the expression of SOSTDC1 plays any roles in carcinogenesis and metastasis of FTC. We found a significantly down-regulated SOSTDC1 expression in follicular thyroid cancer samples. In addition, our data showed that ectopic expression of SOSTDC1 dramatically inhibited thyroid cancer cell proliferation in vitro and in nude mice. SOSTDC1 also compromised the migratory, invasive property, and epithelial–mesenchymal transition (EMT) activity of FTC cell. Mechanically, SOSTDC1 exerted its tumor suppressor function by inhibiting the activity of major signaling pathways including the phosphatidylinositol-3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK)/Erk pathways. Altogether, our findings provide insight into the role of SOSTDC1 as a novel functional tumor suppressor in follicular thyroid cancer through modulating the activities of PI3K/Akt and MAPK/Erk signaling pathways.

Keywords SOSTDC1 · Follicular thyroid cancer · Cell proliferation · Migration · PI3K/Akt · MAPK/Erk

Introduction

Thyroid cancer (TC) is the most prevalent endocrine malignant tumor. Follicular thyroid cancer (FTC) accounts for an estimated 15% of all thyroid cancers cases, and FTC cases tend to have poorer outcomes than the general thyroid cancer cases [1]. Survival rates and life quality of thyroid cancer varies with patient's age, metastasis, and extrathyroid extension. While thyroid cancer itself does not necessarily lead to mortality, minimizing the risk of distant metastasis and extrathyroid extension becomes the major task of cancer management after diagnosis [2]. Currently, surgery, radiotherapy, and chemotherapy are the main treatment therapies for FTC. However, many patients will not respond to maximal surgical and medical therapy [3]. Thus, there is a great need for investigation of the genetic events involved in FTC progression to understand the underlying mechanisms of tumor progression.

In recent years, studies have focused on the proliferation and differentiation activities in thyroid cancer cells. Many molecular pathways were revealed to be related to the growth of thyroid tumor. Among them, the phosphatidylinositol-3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK), originally extracellular signal-regulated kinases (ERK) signaling pathways play an important role in thyroid carcinoma development and progression [4, 5]. PI3K/AKT and MAPK/Erk pathway regulates several cell activities, including cell proliferation, cell differentiation, and cell survival [6–9]. It has been shown that PI3K/Akt and MAPK/Erk pathways are required for induction of epithelial–mesenchymal transition (EMT) [10, 11].

Qinyi Zhou and Jun Chen have been contributed equally to this work and should be considered as equal first coauthors.

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The sclerostin domain containing protein 1 (SOSTDC1) is a cell signaling regulator involved in cell physiology and pathology [12]. Robust evidence has indicated the suppressive effect of SOSTDC1 on various kinds of tumor, including a reported suppression of proliferation of non-small cell lung cancer cells [13] and breast cancer [14]. In this study, this popular protein has caught our attention. We aim to investigate if SOSTDC1 regulate the proliferation of thyroid cancer cells like it does for other cancers, and see if it also affects the malignant properties of tumor cells such cell migration and invasion, and epithelial-mesenchymal transition.

Materials and methods

Clinical samples

Human follicular thyroid cancer (FTC) sample tissues and the adjacent non-cancerous thyroid sample tissues ($n = 10$ pairs) were collected from the RenJi Hospital during total thyroidectomy surgeries for follicular thyroid cancer. The pathological features of the tissue were examined by two pathologists. Samples were only included in the study if they were histologically diagnosed as follicular thyroid cancer. Specimens were snap frozen and stored at −80 °C before use. Written informed consents were obtained from all patients before the study, the study protocol was approved by the Ethic Committee of RenJi hospital.

Cell culture

Human follicular thyroid carcinoma cell line FTC-133 and K1 were purchased from Sigma-Aldrich (Shanghai, China), and cultured with DMEM [Ham's F12 (1:1) + 2 mM glutamine + 10% fetal bovine serum (FBS), Thermofisher] under 37 °C humidified atmosphere with 95% air, 5% CO_2.

SOSTDC1 over-expression via lentiviral transfection

SOSTDC1 was amplified through PCR and subcloned into pCDH-EF1-MCS vector (SBI, Palo Alto, CA, USA). FTC-133 and K1 cells were transfected with SOSTDC1-vector using Lipofectamine 2000 reagent (Thermo Fisher Scientific, China). After 48 h, target gene expression of subjected cells was evaluated by western blot and PCR analysis.

Nude mouse and xenograft transplantation

Athymic nude mice were obtained from the animal center of Shanghai Jiao Tong University at 3 weeks of age and raised until 5 weeks old. Each mouse was subcutaneously injected with 1×10^6 transfected FTC-133 cells at the left side [15]. The study protocol was approved by the animal ethic committee of Shanghai Jiao Tong University.

Western blotting (WB)

Protein of culture cells or clinical samples was extracted using the protein extraction kits (Bio-Rad, Hercules, California, USA) according to the manufacturer's guidance. The protein extracts were loaded on the 12% SDS-PAGE (Bio-Rad, Hercules, California, USA). After electrophoresis, the protein blots of the gels were transferred to PVDF membranes and blocked with non-fat milk (diluted by TBST) for 2 h. The membranes were then subjected to an overnight incubation with primary antibodies at 4 °C, followed by 2 h incubations of the secondary antibody. Antibodies used in this study are as follows: Anti-SOSTDC1 antibody (ab99340), Anti-AKT (ab8805), Anti-p-AKT (ab38449), Anti-ERK1 + ERK2 [ERK-7D8] (ab54230), Anti-ERK 1/2 (phospho-Thr202/Tyr204) (ab214362), Anti-E-Cadherin (ab128804), Anti-SNAIL + SLUG (ab180714), Anti-Vimentin (ab8978) were obtained from Abcam Trading (Shanghai) Company Ltd. Anti-PI3 Kinase p85 (anti-PI3 K) were obtained from the CST (cell signaling technology, Shanghai, China).

Real-time PCR

Real-time quantitative PCR was performed to assess the SOSTDC1 expression levels of thyroid tissues samples and cultured cells [16]. In brief, the whole RNA of samples and culture cells was extracted using the RNA Extraction kits (Thermo Fisher Scientific, Taiwan) according to the manufacturer's instruction. First strand cDNA was generated and amplified by PCR. Real-time quantitative PCR was performed using the LightCycler®480 System (Roche, Indianapolis, IN, USA), GAPDH expression was used as internal control. Target gene expression levels are presented as fold changes relative to GAPDH expression. The PCR primer sequences were summarized as follows: SOSTDC-1: F 5′-GTCGGCTCACAGACAAGTGA-3′, R 5′- CCTCATTAGACGACGTACACG-3′; GAPDH: F 5′-CGGAGTCAACGGATTTGGTCGTAT-3′, R 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′. All PCR experiments were conducted in triplicate samples. The results were interpreted using the $2^{-\Delta\Delta Ct}$ method.

Cell proliferation assessed by MTT assay

An MTT assay was performed to assess cell proliferation [17]. After transfection, cell proliferation was evaluated by

the MTT assay. In brief, the culture cells were prepared at a 96-well plate at 1×10^4 cells/well one day before the test. MTT (20 μM/well) was added to each well and incubate under 37 °C for 4 h. Then the culture medium was removed and DMSO was added. The absorbance was read at 570 nm.

Wound healing assay

A wound healing assay was performed to investigate the potential effect of SOSTDC1 on cell migration [18]. In brief, after transfection, 5×10^5 cells (FTC-133 or K1) were prepared on a 6-well plate and a straight scratch was made for each well (using a pipette tip at an angle of 30°), and then serum culture medium was added and subjected to 37 °C, 5% CO_2 incubation. Cell migration distance was observed at 0 h and 24 h under an optical microscope.

Cell invasion assay

A matrigel assay was performed to assess the cell invasive activity [19]. In brief, after transfection, 5×10^4 cells were added to the chamber (without serum), with 100 μl matrigel (diluted with culture medium, without serum) in the lower chamber. After 24 or 48 h of incubation under 37 °C, the medium was removed and the cells were fixed with formaldehyde for 2 min under room temperature and washed with PBS, and subjected to methanol for 20 min. Finally, the cells were washed and stained with crystal violet for 20 min and observed under optical microscope.

Statistical analysis

All statistical analyses were conducted on SPSS 18.0. Categorical variables are displayed as mean ± SD. Student's t test was performed to determine the statistical difference among datasets and a two-tailed $p < 0.05$ was considered statistical significant.

Table 1 Characteristics of the patients

Patient no.	Age (years)	TNM stage	Sex M/F	Invasiveness
1	35	II	F	M
2	56	II	F	M
3	48	II	M	M
4	55	III	F	M
5	64	III	F	W
6	44	II	F	M
7	66	II	M	M
8	57	II	F	M
9	74	III	M	W
10	68	IV	F	W

M minimally invasive, *W* widely invasive

Results

Down-regulation of SOSTDC1 in follicular thyroid cancer tissue samples

To study the role of SOSTDC1 in follicular thyroid cancer carcinogenesis, we collected ten pairs of follicular thyroid

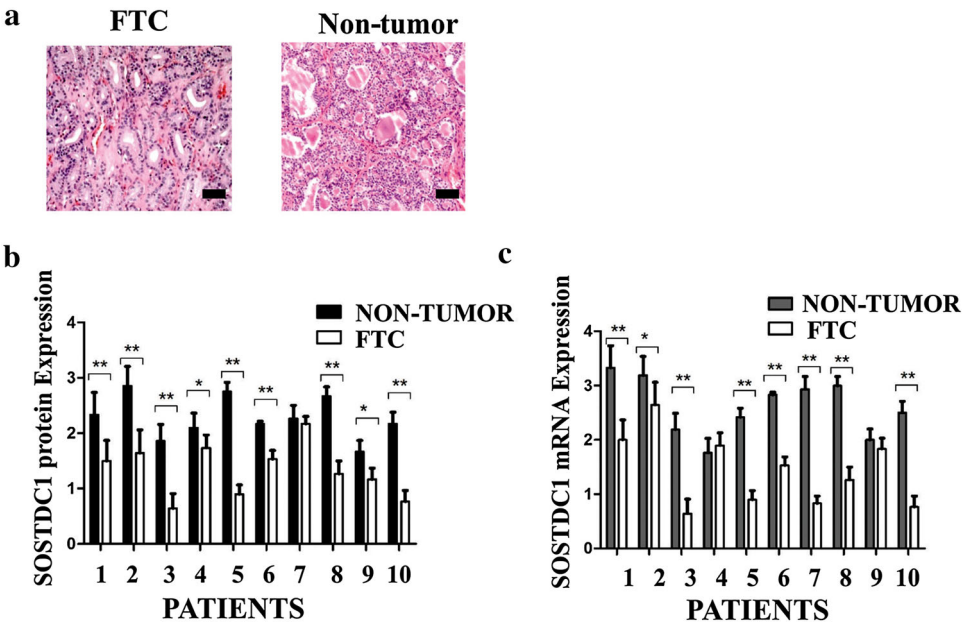

Fig. 1 SOSTDC1 expression of cancerous and adjacent non-cancerous tissues. **a** Representative histological pictures of the cancerous and adjacent non-cancerous tissues, HE-stained, *scale bar* 100 mm; Significantly lower SOSTDC1 expression was observed in western blotting (**b**) and quantitative real-time PCR analysis (**c**), relative to GAPDH. Data are presented as Mean ± SD from three independent experiments. $^{**}p < 0.01$; $^{*}p < 0.05$

carcinoma and the adjacent non-tumor tissue samples (characteristics of the patients are summarized in Table 1). The representative histological pictures of the cancerous and adjacent non-cancerous tissues are shown in Fig. 1a. According to WB and qRT-PCR analysis, we found that SOSTDC1 expression was significantly down-regulated in FTC, compared to the corresponding adjacent non-cancerous tissues (Fig. 1b, c).

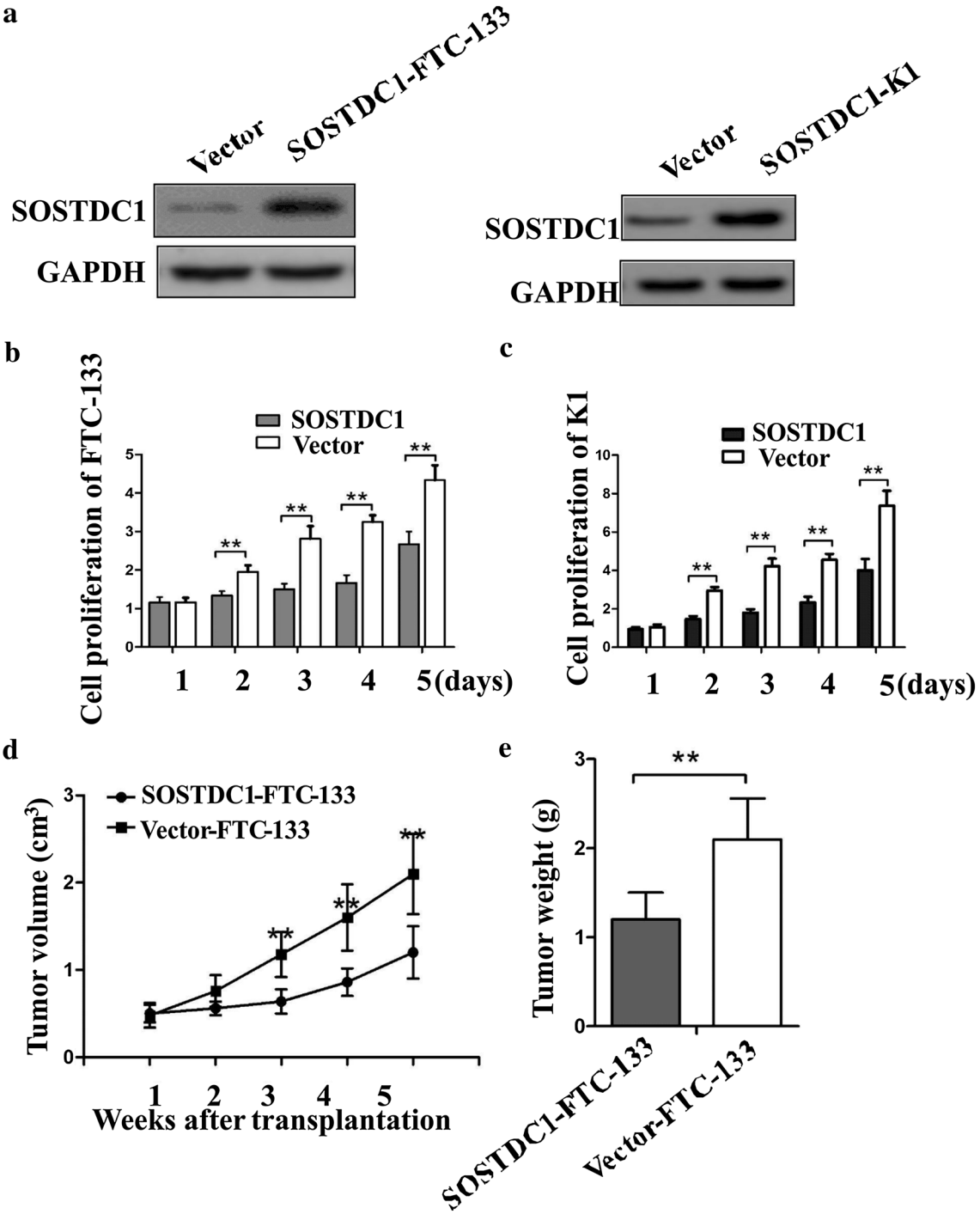

Fig. 2 SOSTDC1 inhibits in vitro and in vivo proliferation of FTC. **a** SOSTDC1-FTC-133 and SOSTDC1-K1 had a stable over-expression of SOSTDC1, compared with the empty-vector control group; In MTT assay, the proliferation of SOSTDC1-FTC-133 and SOSTDC1-K1 was significantly inhibited 2 days after transfection, compared to the control group (**b**, **c**); Data are presented as Mean ± SD from three independent experiments. Tumor growth of SOSTDC1-FTC-133 group was significantly slower after transfection, with significantly lower volume in third, fourth and fifth week (**d**), and a lower value of 5 week tumor weight (**e**). Data are presented as Mean ± SD ($n = 6$ mice per group), $^{**}p < 0.01$; $^{*}p < 0.05$

Inhibition of follicular thyroid cancer cell growth by SOSTDC1

To find out if over-expression of gene SOSTDC1 influences the proliferation of FTC in vitro, we managed to manipulate the SOSTDC1 expression in FTC-133 and K1. SOSTDC1-vector was successfully transfected to FTC-133 and K1 (Fig. 2a). According to the MTT assay, in vitro growth of FTC-133 and K1 was inhibited by SOSTDC1 (Fig. 2b, c).

Inhibition of tumor growth by SOSTDC1 in vivo

To further validate the inhibitive effect of SOSTDC1 on the growth of follicular thyroid tumor in vivo, nude mice were subcutaneously injected with transfected FTC-133 cells. Tumor growth of SOSTDC1-FTC-133-transplanted mice was significantly slower compared to the vector-FTC-133-transplanted mice, with lower values in tumor volumes of third, fourth and fifth, and the 5 week tumor weight (Fig. 2d, e).

Inhibition of follicular thyroid cancer cell migration and invasion by SOSTDC1

Wound healing assay and matrigel assay were performed to investigate if over-expression of SOSTDC1 plays a functional role in cell migration and invasion in thyroid cancer. In the wound healing assay, SOSTDC1-FTC-133 and SOSTDC1-K1 cells showed a significantly less migratory capacity than the control groups (Fig. 3a, b). In the matrigel assay, counts invaded cells in a SOSTDC1-FTC-133 group or SOSTDC1-K1 group were significantly less than their vector control group (Fig. 4a, b). These results suggest an inhibitory effect of SOSTDC1 on the migration and invasion activity of thyroid cancer cells.

SOSTDC1 regulates the activities of PI3K/Akt and MAPK/Erk pathways in follicular thyroid cancer

To find out if SOSTDC1's inhibitory effect on thyroid cancer cell proliferation involves the PI3K/Akt or MAPK/

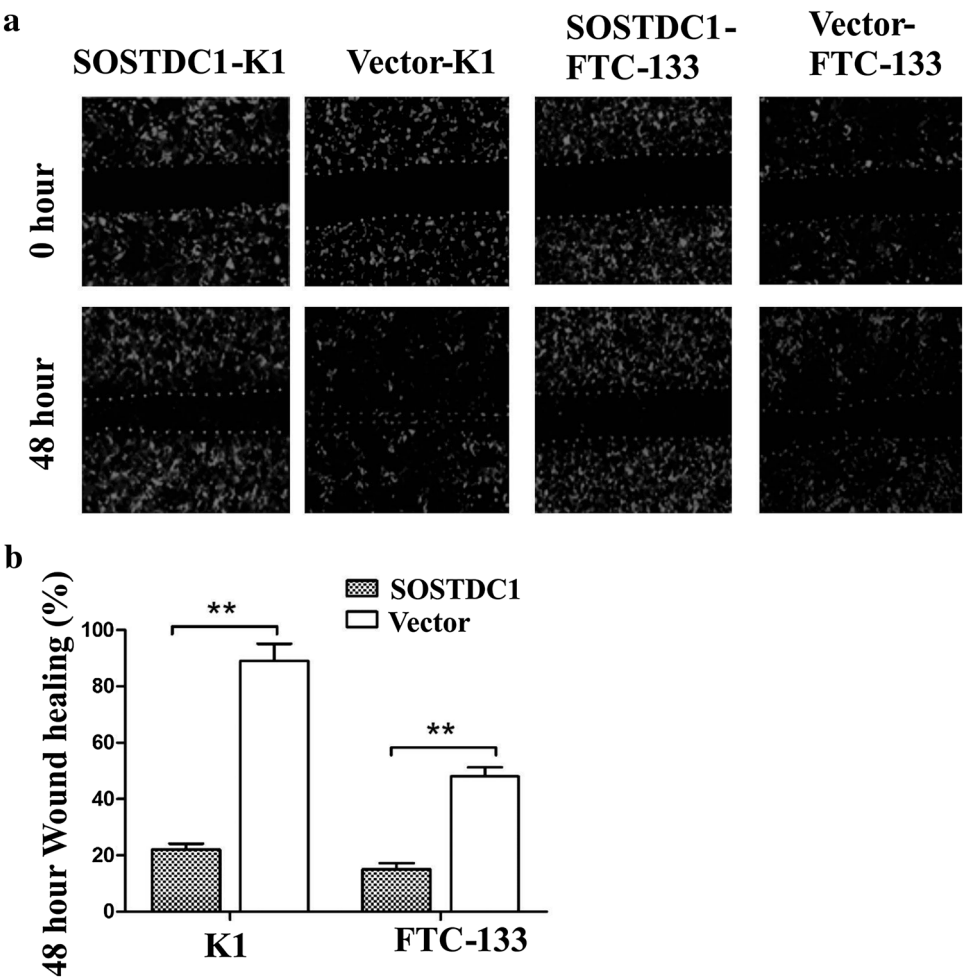

Fig. 3 Wound healing assay of SOSTDC1-Vector transfected cells. **a** Representative pictures of the wound healing assay at 0 and 24 h; **b** quantification of the wound closure percentage; wound closure of SOSTDC1-FTC-133 and the SOSTDC1-K1 group was significantly slower than their vector control groups. Data are presented as Mean ± SD from three independent experiments. $^{**}p < 0.01$; $^{*}p < 0.05$

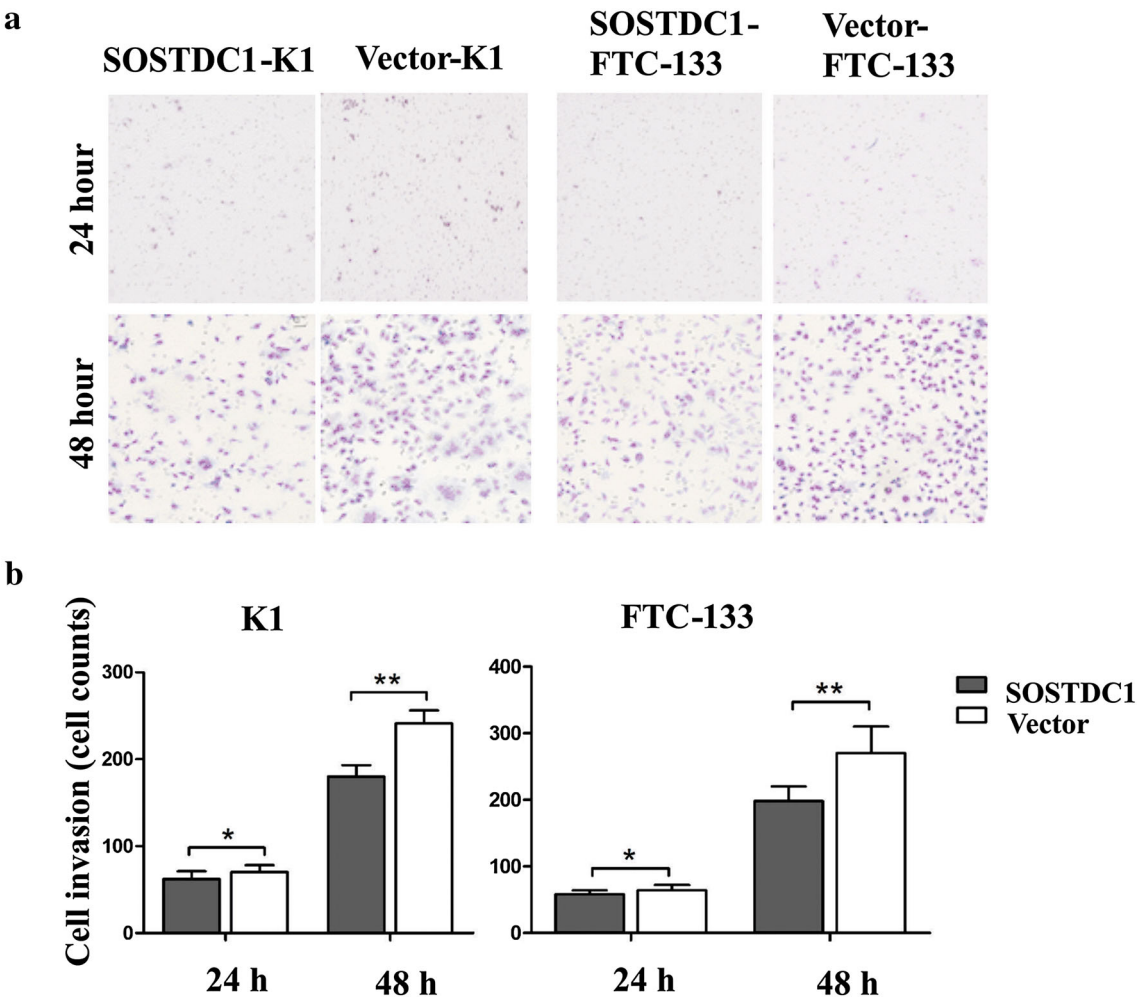

Fig. 4 Cell invasion assay of SOSTDC1-Vector transfected cells. **a** Representative pictures of the matrigel assay at 24 and 48 h; **b** counts of invaded SOSTDC1-Vector transfected cells at 24 and 48 h; SOSTDC1 significantly suppressed the invasive capacity of FTC-133 and K1 cells. Data are presented as Mean ± SD from three independent experiments. $^{**}p < 0.01$; $^{*}p < 0.05$

Erk pathways, Western blotting was performed to assess the expression levels of certain markers, including p-AKT, p-ERK, and PI3K. The result showed that SOSTDC1 caused a significant decrease of p-AKT, PI3K, and p-ERK expression in thyroid cancer cells (FTC-133), compared to the vector control groups. No significant difference was found in AKT or ERK expression (Fig. 5a).

Inhibition of the process of epithelial-mesenchymal transition (EMT) by SOSTDC1

Since the inhibitory effect of SOSTDC1 on the migratory and invasive properties of thyroid cancer cells was confirmed, we would like to know if SOSTDC1 also plays functional roles in epithelial–mesenchymal transition of thyroid cancer cells. Expression of major EMT biomarkers, including E-cadherin, Vimentin, SNAIL, and SLUG, was assessed by western blotting. As demonstrated in Fig. 5b, a significantly lower expression of E-cadherin, Vimentin, SNAIL, and SLUG was observed in SOSTDC1-FTC-133 cells, compared to the vector control group.

Discussion

To our knowledge, this is the first time that direct connections are found between SOSTDC1 and MAPK/Erk-related thyroid cancer cell proliferation and epithelial–mesenchymal transition. In this study, down-regulation of SOSTDC1 was found in follicular thyroid cancer tissue. In order to investigate the functional role of SOSTDC1 in the malignancy of thyroid tumor, we managed to manipulate the expression of SOSTDC1 in thyroid cancer cell line and found that over-expression of SOSTDC1 significantly

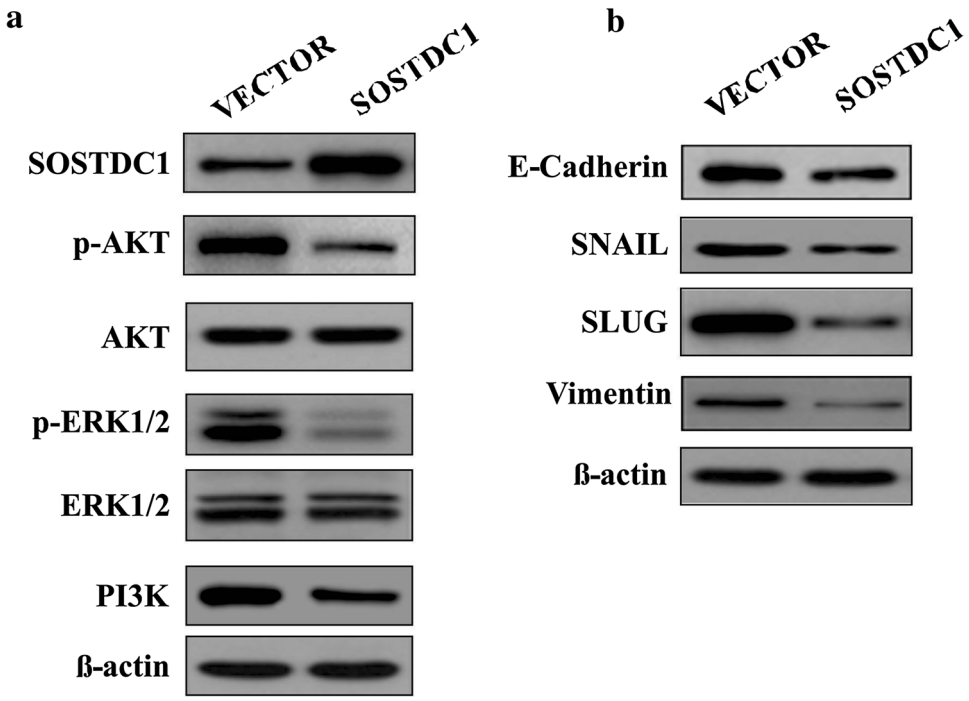

Fig. 5 Change in PI3K/Akt, MAPK/Erk, and EMT markers levels. Expression of SOSTDC1, PI3K, AKT, p-AKT, ERK1/2, p-ERK1/2 (**a**), E-Cadherin, SNAIL, SLUG, and vimentin (**b**) in SOSTDC1-transfected FTC-133 and vector transfected control cells were detected by western blot analysis

inhibited both in vitro and in vivo thyroid tumor growth. We also found an alteration in the PI3K/Akt and MAPK/Erk pathway. In addition, migration and invasion assay also demonstrated SOSTDC1's inhibitory effect on the migratory and invasive potential of thyroid cancer cell. Further, we found significant changes in expression of several epithelial–mesenchymal transition markers.

Recent researches have linked SOSTDC1 to various kinds of cancer, including lung cancer and prostate cancer, and most of these researches concluded a suppressive effect of SOSTDC1 on the cancer growth [13, 20, 21]. In a research of non-small cell lung cancer (NSCLC), SOSTDC1 was found down-regulated in non-small cell lung cancer tissues and over-expression of SOSTDC1 also inhibited the proliferation of NSCLC cells in vitro [13]. In the current study, a similar method was used to investigate the potential role of SOSTDC1 in FTC. We found a significantly lower expression of SOSTDC1 in FTC tissue samples. We managed to manipulate the SOSTDC1 expression in TC cells and found that SOSTDC1 inhibited TC cell proliferation both in vitro and in vivo.

PI3K/Akt and MAPK/Erk signaling pathway plays a vital role in cell survival, apoptosis, cell cycle, and cell migration and is implicated in the pathogenesis of various kind cancers [21–25]. In addition, we also evaluated the PI3K/Akt and MAPK/Erk signaling pathways, which are popular pathways involved in cell growth in TC cells [26, 27], to see if over-expression of SOSTDC1 influences these cell apoptosis-related pathways. Our result suggested that over-expression of SOSTDC1 significantly inhibited the PI3K/Akt and MAPK/Erk pathways of TC cell. The SOSTDC1 protein serves as a bone morphogenetic protein (BMP) antagonist [28]. BMPs play an important role in cell proliferation, apoptosis, and differentiation [29–31] and are also found to be involved in the MAPK/Erk pathways [32]. These facts, along with our observation in this study, suggest that SOSTDC1 protein regulates cell proliferation, possibly through the PI3K/Akt and MAPK/Erk pathways signaling pathways. It is worth mentioning that BMPs are also well known for their roles in epithelial–mesenchymal transition [33–35], a process involved in the initiation of cancer metastasis [36]. We performed cell migration and invasion assay to investigate if SOSTDC1 affects TC cell migration, cell invasion, and epithelial–mesenchymal transition. As we expected, the over-expressed SOSTDC1 significantly inhibits the migratory and invasive activity of TC cells in vitro, and several EMT markers are also found to be down-regulated. Together, we demonstrated the potential of SOSTDC1 in inhibiting thyroid tumor growth and metastasis, which provides a clue for further research of thyroid cancer targeted treatment.

In conclusion, we found a significant down-regulation of SOSTDC1 in thyroid tumor samples. And by manipulation of SOSTDC1 expression in human thyroid cancer cell, we demonstrate the potential of SOSTDC1 in inhibiting thyroid tumor growth and epithelial–mesenchymal transition.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests.

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