Original Article

SQLE induces epithelial-to-mesenchymal transition by regulating of miR-133b in esophageal squamous cell carcinoma

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Abstract

Increasing evidence suggests that microRNAs, which control gene expression at the post-transcriptional level, are aberrantly expressed in cancers and play significant roles in carcinogenesis and cancer progression. In this study, we show differential miR-133b down-expression in human esophageal squamous cell carcinoma (ESCC) cells and tissues. In addition, squalene epoxidase (SQLE), a key enzyme of cholesterol synthesis, is identified as the direct downstream target gene of miR-133b by luciferase gene reporter assay. Furthermore, ectogenic miR-133b expression and SQLE knockdown can inhibit proliferation, invasion, and metastasis, and diminish epithelial-to-mesenchymal transition (EMT) traits of ESCC in vitro, implying that miR-133b-dependent SQLE can induce tumorigenicity and that SQLE is an EMT inducer. Xenograft experiment results also proved the biological function of SQLE in vivo. Therefore, we conclude that miR-133b-dependent SQLE plays a critical role in the potential metastasis mechanisms in ESCC.

Key words: esophageal squamous cell carcinoma, miR-133b, SQLE, epithelial-to-mesenchymal transition

Introduction

Esophageal cancer is the eighth most prevalent malignancy and the sixth most frequent cause of death [1,2]. China is the highest risk area of esophageal cancer and 90% of cases are esophageal squamous cell carcinoma (ESCC) [3]. In particular, the 5-year survival and mortality rates are poor because of its rapid tumor growth, aggressive local invasion and early metastasis [4]. Therefore, understanding the molecular pathogenesis of tumorigenesis of ESCC is extremely important for the discovery of therapeutic targets.

MicroRNAs (miRNAs) are a class of small non-coding RNAs which bind to the 3′-untranslated region (3′-UTR) of mRNAs and degrade them, and act as negative regulators of gene expression at the post-transcriptional level [5-7]. Though considerable evidence has revealed that miRNAs’ expression is commonly dysregulated in human cancers, it is still unclear how miRNAs work to affect tumorigenesis and metastasis. MiRNAs can act as oncogenes or tumor suppressors. For example, miR-200 family members, miR-200a, miR-200b, miR-124, miR-429, and miR-630, were considered as tumor suppressor miRNAs in tumorigenesis [8-12]. However, miR-21, miR-31, miR-192, miR-425, and miR-373 were considered as oncogenes in ESCC [13-17].

Squalene epoxidase (SQLE), located in the endoplasmic reticulum, was found to be the one of the key rate-limiting enzymes in the cholesterol biosynthesis [18,19]. Recent studies have shown that SQLE is involved in the development and metastasis of the tumorigenesis, such as prostate cancers, breast cancer, hepatocellular carcinoma, pancreatic cancer, colorectal cancer, and lung cancer [20-25].

In this study, we found that the expression level of miR-133b was low in ESCC tissues and cells, and ectopic miR-133b expression suppressed the proliferation, invasion, and metastasis of cells. In addition, we identified SQLE as a direct target of miR-133b,
and miR-133b-dependent SQLE knockdown diminished proliferation, invasion, metastasis, and epithelial-to-mesenchymal transition (EMT) traits. Our results suggest that miR-133b/SQLE axis is a potent EMT regulator and plays a significant role in the proliferation and metastasis of ESCC tumorigenesis.

Materials and Methods

Cell lines and reagents
Human esophageal epithelial cell (HEEC) line and human ESCC cell lines KYSE-30, KYSE-70, KYSE-150, and ECA109 were acquired from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). HEEC, KYSE-30, KYSE-70, KYSE-150, and ECA109 cells were all maintained in RPMI 1640 medium (Gibco, Carlsbad, USA) with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO₂.

Patients and specimens
All carcinogenic tissues and matched adjacent normal tissues were obtained with the consents of all patients who were pathologically diagnosed with ESCC at JImin Hospital (Shanghai, China). The Pathology Department of Jimin Hospital selected and preserved these tissues. ESCC diagnosis was defined according to the tumor node metastasis stage classification and World Health Organization (WHO) criteria. This study was approved by the ethics committee of Shanghai Medical Association.

Plasmid construction and cell transfection of oligonucleotides and plasmids
miR-133b mimics (miR-133b) and negative control (miR-NC) were synthesized by Genechem Company (Shanghai, China). SQLE (GenBank No. NM_003129) overexpression plasmid vector was purchased from the Genechem Company and named shSQLE. SQLE short hairpin RNA plasmid vector constructed by Genechem Company was named shSQLE. All of the plasmids were confirmed by DNA sequence analysis. Cells were transfected using the reagent Lipofectamine 2000 (Invitrogen, Carlsbad, USA) in opti-RPMI 1640 (Gibco) according to manufacturer’s instructions.

Microarray analysis
The total RNA was extracted and quantified by the NanoDrop ND-2000 (Thermo Scientific, Waltham, USA). RNA was dephosphorylated by using CIP master mix and labeled with Cyanine-3-CTP (Invitrogen) and exposed to x-ray environment. GAPDH was used as internal references, respectively. Each experiment was carried out in triplicate.

Western blot analysis
The total protein was extracted using RIPA reagent (Beyotime, Nanjing, China) and subject to 10% SDS-PAGE, and then transferred onto the PVDF membranes (Millipore, Boston, USA). The PVDF membranes were blocked with 5% skim milk and then incubated with rabbit antibodies against SQLE, E-cadherin, N-cadherin, Vimentin, and β-catenin (Abcam, Cambridge, UK) or mouse anti-GAPDH antibody (Abcam) for 8 h at 4°C. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit antibody or antimouse antibody (Cell Signaling Technology, Boston, USA). Finally, membranes were detected using an enhanced chemiluminescence kit (Invitrogen) and exposed to x-ray environment. GAPDH was used as the internal reference. Each experiment was carried out in triplicate.

Immunofluorescence staining assay
Cells were seeded on sterilized cover slips and cultured overnight, and then fixed with 4% paraformaldehyde for 20 min, washed with PBS, and incubated with antibodies against E-cadherin, N-cadherin, Vimentin, or β-catenin overnight. Then, cells were incubated with FITC-conjugated goat anti-rabbit secondary antibody (Cell Signaling Technology) for 1 h. After extensive washing, fluorescence staining was examined under a fluorescence microscope (Olympus, Takachiho, Japan). 4′,6-diamidino-2-phenylindole (DAPI) staining was used to visualize cell nuclei.

Dual-luciferase reporter assay
High-fidelity enzyme was used to amplify SQLE putative binding sites of miR-133b. Cells were seeded into the wells of 96-well plates, co-transfected with luciferase reporter plasmids and pSQUE-miR-133b-WT or pSQUE-miR-133b-mut using Lipofectamine 2000. Forty eight hours after transfection, cells were assayed according to the manufacturer’s instructions for the Dual-Luciferase Reporter Assay Kit (Promega, Madison, USA) and the results were read with a SpectraMax M5 microplate reader (Molecular Devices, Silicon valley, USA). Each experiment was carried out in triplicate.

Wound healing assay
Cells were cultured in 24-well plates and transfected as above. A sterile plastic scriber was used to create 1.0-mm wounds when cells overgrew the plate. The cells were washed and incubated in serum-free opti-RPMI 1640 for 24 h. Wound healing was observed under the microscope and images were taken. Each experiment was carried out in triplicate.
MTT assay
Cells were seeded into 96-well plates at 4000 cells per well and cultured for 1–3 days. Then 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) solution (Sigma) was added to the cells at final concentration of 0.5 mg/ml and incubated for 4 h. The remaining MTT formazan crystals were solubilized in 100 μl of DMSO (Sigma) and the OD value of each well was measured with a microplate reader (BioTek, Beijing, China) at 560 nm after removing the medium at 24, 48, and 72 h, respectively. Each experiment was carried out in triplicate.

In vitro Transwell assays
Cell migration and invasion was measured by using Transwell chamber (Corning Co., Corning, USA) with 8.0 μm of pore size and 6.5 mm of diameter. Transfected cells were seeded onto the upper chamber with or without Matrigel (Becton Dickinson and Company, Franklin Lakes, USA) and cultured in serum-free RPMI 1640 medium. Conditioned medium (opti-RPMI 1640 with 4% serum) was added into the lower chambers as chemoattractants. After 24 h, cells in the upper chambers were stained with crystal violet after the medium was removed, and the upper chamber cells were scrapped. Five random fields were selected to record the bottom of the chamber of cells showing invasion and metastasis under the microscope. Each experiment was carried out in triplicate.

In vivo tumor xenografts assay
Six 5-week-old female nude mice with no thymus (First People’s Hospital of Yancheng City, Yancheng, China) were inoculated subcutaneously with 5 × 10^6 ECA109 cells which had been stably transfected with shSQLE or negative control. Mice were randomized into two groups, with three mice in each group. Every 6 days, the tumor volume of each mouse was measured. After 42 days, the mice were sacrificed and the subcutaneous tumors were removed to test the expression levels of EMT molecular markers. All animal experiments complied with the guidelines of the Jiangsu Province Experimental Animal Care and Use Committee.

Statistical analysis
All of the above experiments were repeated at least three times. Categorical variables were compared by using the two-sample tests or Fisher’s exact test where appropriate. The results of in vitro migration assays and luciferase activities were compared by using Student’s t-test. All analyses were performed by using SPSS17.0 statistical analysis software. Experimental data were reported as the mean ± SD. P < 0.05 was considered to be statistically significant.

Results
MiR-133b is remarkably down-regulated in ESCC
To find out the dysregulated miRNA expression profiles in ESCC, miRNA microarray was used. From the result of these miRNA expression profiles, miR-133a and miR-133b were identified to be differentially expressed in ESCC cells compared with HEEC cells (Fig. 1A). Then, qRT-PCR was performed to confirm the result of microarray analysis on miR-133a and miR-133b. The qRT-PCR analysis showed that miR-133b was even conspicuously down-expressed in ESCC tissues and cells (Fig. 1B,C).

MiR-133b suppresses the proliferation, migration, and invasion of ESCC cells
To explored the underlying biological role of miR-133b in ESCC, miR-133b mimics was constructed and transfected into cells. As MTT assays showed, overexpression of miR-133b decreased the proliferation of ESCC cells (Fig. 1D). The wound healing assay revealed that the migratory ability of cells overexpressing miR-133b was significantly lower than that of negative control cells (Fig. 1E). Furthermore, cell migration and invasion was carried out by Transwell assays, and the results demonstrated that overexpression of miR-133b inhibited the migration and invasive ability of ESCC cells (Fig. 1F,G). These data indicated that miR-133b could effectively suppress proliferation, migration, and invasion of ESCC cells.

SQLE is a direct target of miR-133b and SQLE knockdown can inhibit proliferation, migration, and invasion in vitro
To search for the potential downstream targets of miR-133b, three target prediction tools (i.e. TargetScan, miRanda, and miRTarget2) were adopted. They all predicted that SQLE was the direct downstream target of miR-133b.

Moreover, dual-luciferase gene report assay suggested that miR-133b could bind directly to the 3′-UTR of SQLE (Fig. 2A). Transfection with miR-133b could obviously inhibit luciferase activity driven by the wild-type SQLE 3′-UTR, while transfection with miR-NC or the mutant SQLE 3′-UTR could not. Furthermore, the mRNA and protein levels of SQLE were measured when exogenous miR-133b was transfected to investigate the association between miR-133b and SQLE. RT-PCR and western blot assay illustrated that overexpression of miR-133b had lower mRNA and protein level of SQLE compared with the negative control in ESCC cells (Fig. 2B,C). These results suggested that miR-133b suppressed the expression of SQLE by directly targeting the SQLE 3′-UTR in ESCC. Then, SQLE expression in ESCC and normal esophageal squamous tissues were tested by qPCR and results showed that the SQLE expression in ESCC tissues was higher than in the normal esophageal squamous tissues (Fig. 2D).

To understand the biological function of SQLE on ESCC, cells stably transfected with shSQLE were used to perform functional studies. MTT assays revealed that down-expression of SQLE decreased the proliferation of ESCC cells (Fig. 2E). Additionally, the wound healing assay demonstrated that the migratory ability of cells down-expressing SQLE was notably lower than that of negative control cells (Fig. 2F). Meanwhile, cell migration assay was performed by Transwell with Matrigel, and results showed that knockdown of SQLE suppressed the invasive ability of ESCC cells (Fig. 2G). Consistently, cell invasion assay was carried out by Transwell without Matrigel, and a reduction in cell invasion we observed when SQLE was knocked down (Fig. 2H). Taken together, these results illustrated that knockdown of SQLE could prevent ESCC cell progression from proliferation, invasion, and migration.

MiR-133b-dependent SQLE induced proliferation, migration, and invasion in vitro
To identify the biological function of SQLE, which was dependent on miR-133b, we used qPCR and western blotting to detect the change of SQLE expression in ESCC cells co-transfected with anti-miR-133b and shSQLE. It was found that the SQLE expression was up-regulated when the cells were transfected with anti-miR-133b and its expression was obviously down-regulated when cells were co-transfected with
anti-miR-133b and shSQLE (Fig. 3A,B). These phenomena implied that SQLE depends on miR-133b to induce tumorigenicity. 

MTT assays revealed that the proliferation of ESCC cells co-transfected with anti-miR-133b and shSQLE was decreased while the proliferation of cells transfected with anti-miR-133b was increased (Fig. 3C). The wound healing assay results also demonstrated that the migratory ability of cells co-transfected with anti-miR-133b and shSQLE was notably lower, while the migratory ability of cells transfected with anti-miR-133b was higher than the negative control cells (Fig. 3D). Meanwhile, cell migration assay showed that co-transfection with anti-miR-133b and shSQLE suppressed the migration ability of ESCC cells, while transfection with anti-miR-133b
Figure 2. SQLE is a direct target of miR-133b and the effects of SQLE on ESCC cells were evaluated. (A) Relative luciferase activity was analyzed after wild-type or mutant 3′-UTR reporter plasmids were co-transfected with miR-NC or miR-133b into ESCC cells. (B) Confirmation of the expression of SQLE in cell lines by qPCR. (C) Confirmation of the expression of SQLE in cell lines by western blotting. (D) Confirmation of the expression of SQLE in tissues by qPCR. (E) MTT assay was performed to determine cell proliferation. (F) Wound healing was photographed under a microscope at 100× magnification after 6 and 24 h. (G) Transwell migration assay of ESCC cell lines transfected with NC or shSQLE were performed in the 24-well Chambers with Matrigel coating. Cells in five random fields of view were observed at 100× magnification and the invaded cells were counted. (H) Invasion assay of ESCC cell lines transfected with NC or shSQLE. Cells in five random fields of view were observed at 100× magnification and the invaded cells were counted. Each experiment was performed in triplicate. GAPDH was used as an internal control. **P < 0.01.
Figure 3. SQLE is dependent on miR-133b in inducing proliferation, migration, and invasion in vitro

(A) Confirmation of the expression of SQLE in cell lines by qPCR. (B) Confirmation of the expression of SQLE in cell lines by western blotting. (C) MTT assay was performed to determine cell proliferation. (D) Wound healing was photographed under a microscope at 100× magnification after 6 and 24 h. (E) Transwell migration assay of ESCC cell lines transfected with anti-miR-NC, anti-miR-133b, or anti-miR-133b and shSQLE were performed in the 24-well Chambers with Matrigel coating. Cells in five random fields of view were observed at 100× magnification and the invaded cells were counted. (F) Invasion assay of ESCC cell lines transfected with anti-miR-NC, anti-miR-133b, or anti-miR-133b and shSQLE. Cells in five random fields of view were observed at 100× magnification and the invaded cells were counted. Each experiment was performed in triplicate. GAPDH was used as an internal control. **P < 0.01.
induced the migration ability of ESCC cells (Fig. 3E). Consistently, cell invasion assay results also showed a reduction in cell invasion by co-transfection with anti-miR-133b and shSQLE, while an apparent increase in invasion by co-transfection with anti-miR-133b (Fig. 3F). These results illustrated that SQLE was dependent on miR-133b to perform the biological function in ESCC.

EMT was regulated by miR-133b and SQLE in ESCC cells

Recently, EMT has attracted much attention because of its role in regulating metastasis in cancers, including ESCC. The detection of the mRNA and protein levels of EMT molecular markers, including β-catenin, E-cadherin, N-cadherin, and Vimentin, demonstrated that these markers were changed in cells overexpressing miR-133b or down-expressing SQLE.

RT-PCR, western blotting and immunofluorescence assays consistently showed that overexpression of miR-133b significantly increased the expression levels of the epithelial molecular markers such as β-catenin and E-cadherin, whereas notably decreased the expression levels of the mesenchymal molecular markers such as N-cadherin and Vimentin, when compared with the negative control (Fig. 4A–C).

Meanwhile, RT-PCR, western blotting, and immunofluorescence assays consistently showed that SQLE down-expression significantly increased the expression levels of the epithelial molecular markers including β-catenin and E-cadherin, while notably decreased the expression levels of the mesenchymal molecular markers including N-cadherin and Vimentin, when compared with the negative control (Fig. 5A–C).

These results together illustrated that ectogenic miR-133b expression or knockdown of SQLE could stabilize the epithelial phenotype of ESCC cells and modulate EMT processing in ESCC progression.

SQLE knockdown suppressed tumor growth and EMT in athymic nude mice

Tumors in nude mice were generated by ECA109 cells stably transfected with shSQLE or negative control. After 42 days, mice were sacrificed and the tumor volumes were measured. Tumor sizes of nude mice inoculated with shSQLE had smaller tumor volumes and slower growth rates compared with the negative control (Fig. 6A,B). qPCR assay indicated that the subcutaneous tumors generated with shSQLE had a higher expression of E-cadherin and β-catenin but a lower expression of N-cadherin and Vimentin compared with the negative control (Fig. 6C). These data suggested that knockdown of SQLE could block the ability of proliferation, migration, and invasion to influence the process of EMT of ESCC in vivo.

Discussion

ESCC has been one of the most lethal popular malignancies in the Southeast Asia [26]. Due to its aggressive invasion and easy metastasis, it is difficult to treat the patients [27]. However, with the progress of the medicine and the deepening of the research about the mechanisms of tumorigenesis and metastasis on various cancers, especially on ESCC, more and more evidence has been reported that the dysregulation of some specific oncogenes and/or anti-oncogenes in multiple complicated signal pathways is related to the metastasis of ESCC [28,29]. For instance, PITX2 activated the Akt/GSK-3β/β-catenin signaling pathway by being targeted by miR-644a to promote the ESCC aggressiveness and stem-cell-like phenotype [30]. DNMT1 was involved in methylation of tumor suppressors and improved proliferation, metastasis, and invasion in SCC and down-expression of DNM1T1 suppressed the methylation of RASSF1 and DAPK to inhibit the invasion and metastasis [31]. NDRG1 was shown to be a pro-oncogenic gene which regulates the Wnt pathway and EMT by affecting TLE2 in ESCC [32]. Nevertheless, the exact mechanisms of biological molecules on ESCC invasion and metastasis have not been identified. A better understanding of the molecular networks in the early invasion and metastasis can help us to cure ESCC.

MiRNAs are small important non-coding RNAs involved in post-transcriptional regulation [5]. Disregulation of miRNA expression commonly occurs in human cancers including esophageal cancers, and the expression profiling was relevant to progression, proliferation, invasion, metastasis, apoptosis, and prognosis [5,33,34]. MiR-365 and miR-129 expression levels in ESCC patients can be used as non-invasive way for prediction [35]. MiR-306 could serve as potential biomarkers for the diagnosis and prognostic prediction of ESCC [36]. MiR-495 acts as a tumor suppressor which inhibits cell-cycle transition and the EMT signaling pathway through targeting Akt1 in ESCC [37]. However, it is still not clear how miRNAs promote or suppress tumorigenesis or via what pathways miRNAs regulate the invasion and metastasis of ESCC. It is extremely important to understand the carcinogenesis of ESCC.

In our previous study, we observed that miR-133b was down-expressed in ESCC cells. Furthermore, ectopic miR-133b transfection could suppress the proliferation, invasion, and metastasis of cells. Similarly, Fu et al. [38] reported that miR-133b was down-regulated in patients with ESCC from China. Bioinformatics analyses revealed that miR-133b was involved in invasion and metastasis of ESCC. In addition, Chen et al. [39] demonstrated that miR-133b was down-regulated in ESCC tissues and positively correlated with tumor stage and differentiation. Moreover, miR-133b might improve the response rate of ESCC patients to paclitaxel-based chemosensitivity and predict prognosis for postoperative overall survival in ESCC patients. In addition, Kano et al. [40] found that miR-133b was down-regulated in ESCC tissues and it directly targeted FSCN1 to contribute to the decrease of cellular proliferation and invasion in ESCC.

SQLE is an important enzymes located in the endoplasmic reticulum. It is involved in cholesterol biosynthesis, and catalyzes the conversion of squalene to 2,3(S)-oxidosqualene. Recently, dysregulation of SQLE was proposed to be associated with the molecular pathogenesis in various cancers [18,41]. There was high SQLE copy number and gene expression in breast cancer and high SQLE expression was an independent prognostic factor of unfavorable outcome. Down-expression of SQLE was less prevalent in aggressive breast cancer and could decrease cell viability and amplification, but increase the replication time [21]. Another study on breast cancers revealed that SQLE was differentially expressed and seemed to exist from the earliest stages of tumor development [42]. Myc gene amplification and aberrant DNA methylation of SQLE promoter have also been observed in aggressive breast cancer [43]. Interaction of SQLE and fibroblasts possibly could affect the carcinoma phenotype. Recently, some researchers explained that SQLE was up-regulated in the hepatocellular carcinoma tissues and SQLE could promote the proliferation and migration in vitro and in vivo via positively regulating the ERK signaling pathway [22]. In lung cancer, SQLE expression served as a molecule and poor prognosis marker because of a high expression of SQLE in tissues, which was closely related to the occurrence and development of squamous cell lung carcinoma [44]. Similar biological functions also exist in colorectal cancer and ovarian cancer [24,45]. Microarray analysis indicated that SQLE was associated with radioreistance in the pancreatic cancer cell lines, and SQLE worked as an
Figure 4. EMT is regulated by miR-133b in ESCC cells

(A) Confirmation of the expression of epithelial markers (E-cadherin and β-catenin) and mesenchymal markers (N-cadherin and Vimentin) in ESCC cell lines by qPCR. (B) Confirmation of the expression of epithelial markers (E-cadherin and β-catenin) and mesenchymal markers (N-cadherin and Vimentin) in ESCC cell lines by western blotting. (C) Confirmation of the expression of epithelial markers (E-cadherin and β-catenin) and mesenchymal markers (N-cadherin and Vimentin) in ESCC cell lines by immunofluorescence staining. DAPI was used to visualize nuclei. GAPDH was used as an internal control. Each experiment was performed in triplicate. **P < 0.01.
oncogene that altered cell-cycle progression signature, induced migration and invasion, and predicted poor prognosis in prostate cancer [23,46]. High SQLE expression increased angiogenesis which facilitates micrometastatic, and established poor prognostic for prostate cancer with or without prostatectomy. In addition, ductal carcinoma of the prostate had a different immunophenotype with change of steroid synthesis-related enzymes [20]. However, the biological function of SQLE in ESCC is unknown. In this study, we identified SQLE as a
direct target of miR-133b by using dual-luciferase gene report, qPCR and western blot assay. Disrupting SQLE diminished the ESCC cells proliferation, invasion and metastasis in vitro. We further defined the miR-133b-dependent SQLE that induced tumorigenicity in ESCC.

EMT has been regarded as epithelial cells acquire the invasive motile properties for metastases [47]. In EMT, epithelial phenotype cells lose cell polarity and proliferative control, degrade the extracellular matrix, and transform into mesenchymal phenotype cells [48,49]. These events enhance angiogenesis and distant metastasis. In the present study, we assessed the expression levels of epithelial phenotype and mesenchymal phenotype proteins in ESCC cells with disrupted SQLE. Notably, the expression levels of the epithelial markers E-cadherin and β-catenin were dramatically increased, while the expression levels of the mesenchymal markers N-cadherin and Vimentin were decreased in ectogenic miR-133b and disrupted SQLE ESCC cells. These data support that there is a relationship between miR-133b, SQLE, and EMT-related transformation in ESCC.

Taken together, our results reveal that miR-133b expression is down-regulated, consistent with the previous studies in ESCC. Additionally, we illustrate that ectogenic miR-133b expression inhibits cell proliferation, migration and invasion capabilities by directly targeting SQLE in ESCC cells. Furthermore, our results identify that SQLE is dependent on miR-133b in inducing proliferation, invasion, and EMT in vivo and in vitro. This novel signal axis of ESCC may provide a new insight into the potential tumor suppressive mechanisms of ESCC initiation and progression. Our study supports a critical role of SQLE in ESCC tumorigenesis, and the miR-133b/ SQLE axis may be a promising therapeutic strategy.

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