**ORIGINAL ARTICLE**

**Circ_0001273 downregulation inhibits the growth, migration and glutamine metabolism of esophageal cancer cells via targeting the miR-622/SLC1A5 signaling axis**

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**Abstract**

**Background:** Esophageal cancer is a relatively rare cancer. However, its death rate is not to be taken lightly. Accumulating evidence indicates circular RNA (circRNA) is implicated in cancer development. The objective of this study was to unveil the role of circ_0001273 in esophageal cancer (EC).

**Methods:** For expression analysis of circ_0001273, miR-622 and solute carrier family 1 member 5 (SLC1A5), quantitative real-time PCR (qPCR) and Western blot were conducted. Cell proliferation was evaluated by cell counting kit-8 (CCK-8), EdU and colony formation assays. Cell apoptosis and cell migration were investigated using flow cytometry assay and wound healing assay. Glutamine metabolism was assessed by glutamine consumption and glutamate production using matched kits. The predicted binding relationship between miR-622 and circ_0001273 or SLC1A5 was validated by dual-luciferase reporter assay. An in vivo xenograft model was established to determine the role of circ_0001273 on tumor growth.

**Results:** Circ_0001273 was upregulated in EC tumor tissues and cells. Knockdown of circ_0001273 repressed EC cell proliferation, migration, epithelial-mesenchymal transition (EMT) and glutamine metabolism. Circ_0001273 knockdown also blocked tumor development in animal models. MiR-622 was targeted by circ_0001273, and its inhibition reversed the functional effects of circ_0001273 knockdown. SLC1A5 was a target gene of miR-622, and circ_0001273 targeted miR-622 to positively regulate SLC1A5 expression. The inhibitory effects of miR-622 enrichment on EC cell proliferation, migration, EMT and glutamine metabolism were recovered by SLC1A5 overexpression.

**Conclusion:** Circ_0001273 high expression contributed to EC progression via modulating the miR-622/SLC1A5 signaling axis.

**KEYWORDS**
circ_0001273, esophageal cancer, miR-622, SLC1A5

**INTRODUCTION**

Esophageal cancer (EC) ranked seventh in cancer-related incidence and sixth in cancer-related mortality in 2020.\(^1\) Esophageal cancer is the most aggressive of all gastrointestinal malignancies, with esophageal squamous cell carcinoma (ESCC) being the predominant type.\(^2\) The global 5-year overall survival rate is 15% to 25%, mainly due to late diagnosis and propensity to metastasize.\(^3\) As standard treatment remains limited to surgical or endoscopic resection and...
Radiotherapy, a better understanding of the molecular pathogenesis of EC is needed.

Novel evidence implicates circular RNAs (circRNAs) in the pathogenesis of multiple cancers. CircRNA refers to a class of non-coding RNAs with a loop-closed structure, thus harboring no 5’ and 3’ ends. CircRNA is highly conserved, showing tissue-, cell type- or developmental stage-specific expression patterns, relating to multiple biological functions in physiological processes. CircRNA has been proposed as a useful disease biomarker due to its high stability, specific expression, and presence in a variety of body fluids. With the wide presence of circRNA expression profiles, plenty of circRNAs have been recognized to be aberrantly expressed in EC tumor tissues. In detail, the specific involvement of numerous circRNAs in EC biology has been unveiled. For example, circ_0000654 was abundantly expressed in ESCC, relating to tumor stage and metastasis, and circ_0000654 depletion impaired ESCC cell growth, survival, migration and invasion. A circRNA microarray that identified the differently expressed circRNAs in plasma of ESCC patients was uploaded onto the GEO database (accession: GSE112496). Herein, we analyzed the data from this dataset and identified that hsa_circRNA_406237 (also named circ_0001273) was overexpressed in plasma of ESCC patients, suggesting the potential involvement of circ_0001273 in EC development. Circ_0001273 is produced from oxidoreductase NAD binding domain containing 1 (OXNAD1) mRNA via “back-splicing.” However, the functional role of this circRNA has not been expounded in EC.

CircRNA exerts multifaceted biological mechanisms, such as acting as a microRNA (miRNA) sponge. Currently, the development of several circRNA-specific bioinformatic databases, such as Circinteractome and circBank, are easily used to predict circRNA-targeted miRNAs. MiR-622 is a well-demonstrated tumor suppressor in multiple cancers, including EC. It has also been predicted as a potential target of circ_0001273, whereas, the implication between circ_0001273 and miR-622 in EC development remains unclear. Of note, several oncogenes have been identified to be targets of miR-622, such as vascular endothelial growth factor A (VEGFA) and Kirsten rat sarcoma (KRAS). Accordingly, miR-622 targets these oncogenes to inhibit the progression of cancers. However, there are still numerous target genes potentially targeted by miR-622 that have not been confirmed. Using bioinformatic prediction, solute carrier family 1 member 5 (SLC1A5) was predicted as a target of miR-622. The oncogenic role of SLC1A5 in EC attracted our attention. We thus studied the interplay between miR-622 and SLC1A5 in EC development.

Our study validated the expression of circ_0001273 in EC tumor tissues and cell lines and conducted loss-function experiments to investigate the functions of circ_0001273 in vitro and in vivo. Crucially, we established circ_0001273-mediated miR-622/SLC1A5 pathway to expound its regulatory mechanism, aiming to illustrate the novel molecular pathogenesis of EC.

**METHODS**

**Clinical tissues**

Twenty-nine pairs of esophageal tumor tissues and matched normal tissues were removed from patients during surgery, with their written informed consent. The pathological diagnosis of tumors was previously accomplished by two pathologists. Patients were recruited from Gaozhou People’s Hospital, and patients with other malignancies were excluded. All samples were surgically excised, frozen in liquid nitrogen and preserved in a −80°C environment. The implementation of this study was approved by the Ethics Committee of Gaozhou People’s Hospital.

**Immunohistochemistry (IHC) assay**

Tumor tissues and normal tissues from patients were embedded into paraffin, and then 4-μm tissue sections were prepared. Tissue sections were subjected to deparaffinization, rehydration and antigen retrieval. Subsequently, tissue sections were probed with the primary antibodies against Ki67 (ab15580; Abcam). After incubating tissue sections with secondary antibody, sections were stained with diaminobenzidine (DAB) kit (Beyotime). High abundance of Ki67 was used to further identify tumor tissues and normal tissues. In addition, tumor tissues from mice were also embedded into paraffin to prepare 4-μm tissue sections. The procedures were conducted as abovementioned. The primary antibodies included anti-E-cadherin (ab40772; Abcam), anti-N-cadherin (ab76011; Abcam) and anti-SLC1A5 (ab237704; Abcam).

**Cell lines**

Human esophageal epithelial cells (HEEC, control) and EC cell lines (KYSE30, KYSE180, TE1 and ECA109) were purchased from BeNa cell bank (Beijing, China). HEEC and ECA109 cells were maintained in DMEM and supplemented with 10% FBS. KYSE30, KYSE180 and TE1 cells were cultured with RPMI1640 medium and supplemented with 10% FBS. An incubator at 37°C supplemented with 5% CO2 was used to culture these cell lines.

**Quantitative real-time PCR (qPCR)**

Trizol reagent (Cwbio) was employed to extract total RNA, followed by quantification under a microspectrophotometer (Thermo Fisher). Total RNA was reverse transcribed into cDNA using QuantiTect reverse transcription kit (Qiagen) or miRNA 1st Strand cDNA synthesis kit (Vazyme). Thereafter, cDNA was quantified using UltraSYBR mixture (Cwbio), using β-actin or U6 to normalize the expression. Primers used for quantitative real-time (qPCR) are listed in Table 1.
RNase R digestion assay

Total RNA extracted from TE1 and ECA109 cells was exposed to RNase R (BioVision) for 30 min at 37°C and then used for qPCR to detect the expression of circ_0001273, using GAPDH as a linear molecular control.

Cell transfection

Short-hairpin RNA targeting circ_0001273 (sh-circ_0001273, 5′-ATAAAGAGATTGTGTCAGCAG-3′) and matched negative control (sh-NC) were designed by Geneseed. Mimic of miR-622 (miR-622, 5′-ACAGCAGGCAAGAGGCAAGU3′), inhibitor of miR-622 (anti-miR-622, 5′-ACUGCCU GUGCUGUCCUCU3′) and their corresponding negative control (6-NC and anti-miR-NC, 5′-UUCUCGGAGCGUGCACGTCTG3′ and 5′-ACUGCCU CUGAGGACGUAUA3′) were directly purchased from Ribobio. pcDNA expression vector was used for SLC1A5 overexpression, and fusion pcDNA-SLC1A5 vector (SLC1A5) and pcDNA empty vector control (pcDNA) were provided by Genepharma. These oligonucleotides or vectors were transfected into TE1 and ECA109 cells using lipofectamine 3000 reagent (Invitrogen).

Cell counting kit-8 (CCK-8) assay

The treated cells were seeded into 96-well plates (5 × 10^3 cells/well), in triplicate. Cells were incubated for 48 h and next treated with CCK-8 reagent (10 μl in each well) for another 2 h. To assess cell viability, optical density (OD) value (450 nm) was measured using a microplate reader (BioTek).

EdU assay

Cell proliferation was investigated using a Cell-Light EdU Apollo567 in vitro kit (Ribobio) in accordance with the manufacturer’s instructions. After culturing cells with EdU solution, cells were fixed with 4% paraformaldehyde, and the cell nuclei were stained with DAPI. The images of EdU-positive cells were captured and the number of cells was counted under a light microscope (Nikon).

Colonies formation assay

The treated cells were plated into 6-well plates (200 cells/well), in triplicate. Cells were maintained in a 37°C incubator supplemented with 5% CO2 for over 10 days. Afterwards, cell debris was washed with PBS, and colonies were fixed with methanol and stained with crystal violet. Images were taken using a Nikon camera.

Flow cytometry assay

According to the protocol of annexin V-FITC/PI apoptosis detection kit (Vazyme), the treated cells cultured for 48 h were collected in annexin V-FITC binding buffer. Next, annexin V-FITC and propidium iodide (PI) were used to stain cells in the dark. The apoptotic cells were sorted and analyzed by flow cytometry (Beckman).

Wound healing assay

The treated cells were cultured for 24 h, then plated into 24-well plates and cultured overnight. An artificial wound was created on the cell surface using a sterile pipette tip. Images of wound distance were immediately taken under a microscope (Nikon; 40×). After culturing cells for 24 h, images of wound distance were taken again. Then, the migration distance was assessed using Image J software.

Western blot assay

EMT-related markers (E-cadherin and N-cadherin) and SLC1A5 protein were quantified by Western blot as previously described. The primary antibodies used including anti-E-cadherin (ab40772), anti-N-cadherin (ab76011), anti-SLC1A5 (ab237704) and anti-β-actin (ab8227), were obtained from Abcam.

Glutamine consumption and glutamate production assay

Commercial detection kits, including glutamate assay kit (Colorimetric) (Abcam) and glutamate assay kit (Abcam), were utilized to detect glutamine consumption and...
glutamate production in cell culture medium according to matched protocols.

Dual-luciferase reporter assay

The target miRNAs of circ_0001273 and their binding sites were predicted by Circinteractome (https://circinteractome.nia.nih.gov/) and CircBank (http://www.circbank.cn/). The target genes of miR-622 and their binding sites were predicted by TargetScan (http://www.targetscan.org/vert_72/). According to the putative binding sites between miR-622 and circ_0001273 or SLC1A5 3'UTR, the wild-type (WT) and mutant-type (MUT) reporter plasmids of circ_0001273 or SLC1A5 3'UTR were constructed utilizing pmirGLO plasmid. To validate their binding sites, WT-circ_0001273 or MUT-circ_0001273 and miR-622 or miR-NC were transfected into TE1 and ECA109 cells. Likewise, WT-SLC1A5 3'UTR or MUT-SLC1A5 3'UTR and miR-622 or miR-NC were also transfected into TE1 and ECA109 cells. After culturing for 48 h, luciferase activity was checked in cells using a dual-luciferase reporter assay system (Promega).

Xenograft model assay

The experimental nude mice (Balb/c, female) were purchased from Beijing Vital River Animal Co., Ltd. Sh-circ_0001273 or sh-NC was subjected to lentivirus packaging by Geneseed. Sh-circ_0001273 or sh-NC virus particles were used to infect ECA109 cells to construct stable expression cell lines. Next, the infected ECA109 cells were subcutaneously implanted into nude mice (n = 6 per group). During tumor formation, tumor volume (length × width² × 0.5) was measured every 3 days using a caliper. Finally, after the tumors had been allowed to grow for 23 days, all mice were killed and tumor tissues were excised. The operation of the animal study was approved by the Animal Care and Use Committee of Gaozhou People’s Hospital.
Statistical analysis

All experiments were independently conducted three times. Experimental data were processed by GraphPad Prism 7.0 (GraphPad). Statistical analysis, such as difference comparison in diverse groups, was conducted by Student’s t-test or analysis of variance. The correlation between miR-622 expression and circ_0001273 expression or SLC1A5 expression in tumor tissues was analyzed by Pearson’s analysis. Data are expressed as the mean ± standard deviation, and p-values less than 0.05 were considered statistically significant.

RESULTS

Circ_0001273 was overexpressed in EC tumor tissues and cell lines

To confirm the characteristics of tumor tissues, an IHC assay was performed to examine Ki67 expression in both tumor and normal tissues. As shown in Figures 1a,b, high expression of Ki67 was exhibited in tumor tissues relative to normal tissues.

Knockdown of circ_0001273 suppressed EC cell malignant phenotypes

Given that circ_0001273 expression was relatively higher in TE1 and ECA109 cells (Figure 1f), TE1 and ECA109 cells

Data from the GSE112496 dataset confirmed that hsa_circRNA_406237 (circ_0001273) was one of the upregulated circRNAs in the plasma of ESCC patients by microarray analysis (Figure 1c). Consistently, we observed that circ_0001273 was also forcefully expressed in our clinical tumor tissues relative to normal tissues (Figure 1d). The receiver operating characteristic (ROC) curve showed that circ_0001273 had a considerable diagnostic value, with the areas under the ROC curve (AUC) = 0.8276 (Figure 1e). The expression of circ_0001273 was also notably reinforced in KYSE30, KYSE180, TE1 and ECA109 cells compared with that in HEEC cells (Figure 1f). Further analysis illustrated that circ_0001273, compared to GAPDH, was largely resistant to RNase R digestion, which verified the reality of circ_0001273 (Figure 1g,h). The data showed the aberrant expression of circ_0001273 in EC.
FIGURE 3 MiR-622 was identified as a target of circ_0001273. (a) The potential targets of circ_0001273 were predicted by Circinteractome and CircBank. (b, c) The expression of miR-432-5p, miR-507 and miR-622 in TE1 and ECA109 cells with circ_0001273 knockdown was measured by quantitative real-time PCR (qPCR). (d) The predicted binding sites between circ_0001273 and miR-622. (e) The efficiency of miR-622 mimic was checked by qPCR. (f, g) The predicted binding sites between circ_0001273 and miR-622 were validated by dual-luciferase reporter assay. (h) MiR-622 expression in tumor tissues and normal tissues was measured by qPCR. (i) The correlation between miR-622 expression and circ_0001273 expression in tumor tissues. (j) MiR-622 expression in HEEC, TE1 and ECA109 cells was measured by qPCR. ***p < 0.01; ****p < 0.001.

FIGURE 4 MiR-622 inhibition reversed the effects of circ_0001273 knockdown. In TE1 and ECA109 cells transfected with sh-circ_0001273 alone or sh-circ_0001273 + anti-miR-622, (a) the expression of miR-622 was measured by qPCR. (b–d) Cell proliferation was determined by cell counting kit-8 (CCK-8) assay, EdU assay and colony formation assay. (e) Cell apoptosis was ascertained by flow cytometry assay. (F) Cell migration was determined by wound healing assay. (g, h) The expression levels of E-cadherin and N-cadherin were measured by Western blot. (i, j) Glutamine consumption and glutamate production was determined by using matched kits. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.
transfected with sh-circ_0001273 or sh-NC were used in the following assays. Circ_0001273 expression was markedly declined in TE1 and ECA109 cells after sh-circ_0001273 transfection (Figure 2a). Circ_0001273 deficiency largely impaired cell viability, reduced the number of EdU-positive cells, and decreased the number of colony formation, by CCK-8, EdU and colony formation assays, respectively (Figures 2b–d). In addition, flow cytometry assay confirmed that the apoptotic rate was remarkably promoted in TE1 and ECA109 cells with circ_0001273 knockdown (Figure 2e,f). By wound healing assay, we monitored that circ_0001273 downregulation weakened TE1 and ECA109 cell migration distance (Figure 2g). Moreover, we noticed that E-cadherin expression was reinforced, while N-cadherin expression was weakened in TE1 and ECA109 cells transfected with sh-circ_0001273 (Figure 2h, i). Circ_0001273 downregulation also inhibited glutamine consumption and glutamate production, thus repressing glutamine energy metabolism (Figure 2j,k). These data revealed that circ_0001273 knockdown inhibited EC cell proliferation, migration, EMT and glutamine metabolism.

Circ_0001273 directly targeted miR-622

A total of three miRNAs were commonly predicted using Circinteractome and CircBank (Figure 3a). We then examined their expression in TE1 and ECA109 cells after circ_0001273 knockdown and discovered that miR-622 showed the highest expression in TE1 and ECA109 cells with circ_0001273 knockdown (Figure 3b,c). To verify the binding sites between circ_0001273 and miR-622, dual-luciferase reporter assay was performed. The WT and MUT constructs of circ_0001273 are shown in Figure 3d. The transfection of miR-622 mimic markedly enriched the expression of miR-622 in TE1 and ECA109 cells (Figure 3e). Next, miR-622 enrichment remarkably diminished luciferase activity in TE1 and ECA109 cells transfected with WT-circ_0001273 rather than MUT-circ_0001273 (Figure 3f,g). The expression of miR-622 was strikingly decreased in tumor tissues relative to normal tissues (Figure 3h), and a negative correlation existed between miR-622 expression and circ_0001273 expression in tumor tissues (Figure 3i). Likewise, miR-622 expression was also lower in TE1 and ECA109 cells than that in HEEC cells (Figure 3j). MiR-622 showed an opposite expression pattern with circ_0001273 and was confirmed to be a target of circ_0001273.

Inhibition of miR-622 reversed the role of circ_0001273 knockdown and recovered EC cell malignant phenotypes

We conducted rescue experiments to further verify the interaction between circ_0001273 and miR-622 in cell functions. The expression of miR-622 enhanced in TE1 and
ECA109 cells transfected with sh-circ_0001273 was substantially repressed in cells transfected with sh-circ_0001273 + anti-miR-622 (Figure 4a). In function, circ_0001273 knockdown-reduced cell viability, the number of EdU-positive cells and the number of colony formation were considerably restored by further miR-622 inhibition (Figure 4b–d). Circ_0001273 knockdown-induced cell apoptosis was largely ameliorated by miR-622 repression (Figure 4e). The inhibitory cell migration distance by circ_0001273 knockdown was also recovered by miR-622 inhibition (Figure 4f). The enhanced expression of TE1 and the inhibitory expression of ECA109 in TE1 and ECA109 cells caused by circ_0001273 knockdown were largely reversed by further miR-622 inhibition (Figure 4g,h). Moreover, glutamine consumption and glutamate production suppressed by circ_0001273 downregulation were remarkably recovered by additional miR-622 inhibition (Figure 4i, j). Rescue experiments indicated that circ_0001273 knockdown inhibited EC cell malignant phenotypes via increasing miR-622 expression.

SLC1A5 identified as a target of miR-622

The binding site between miR-622 and SLC1A5 3’UTR was predicted by TargetScan, and the WT and MUT constructs of SLC1A5 3’UTR are shown in Figure 5a. MiR-622 enrichment remarkably weakened luciferase activity in TE1 and ECA109 cells transfected with sh-circ_0001273 or sh-circ_0001273 + anti-miR-622 was measured by Western blot. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

ECA109 cells transfected with sh-circ_0001273 was substantially repressed in cells transfected with sh-circ_0001273 + anti-miR-622 (Figure 4a). In function, circ_0001273 knockdown-reduced cell viability, the number of EdU-positive cells and the number of colony formation were considerably restored by further miR-622 inhibition (Figure 4b–d). Circ_0001273 knockdown-induced cell apoptosis was largely ameliorated by miR-622 repression (Figure 4e). The inhibitory cell migration distance by circ_0001273 knockdown was also recovered by miR-622 inhibition (Figure 4f). The enhanced expression of TE1 and the inhibitory expression of ECA109 in TE1 and ECA109 cells caused by circ_0001273 knockdown were largely reversed by further miR-622 inhibition (Figure 4g,h). Moreover, glutamine consumption and glutamate production suppressed by circ_0001273 downregulation were remarkably recovered by additional miR-622 inhibition (Figure 4i, j). Rescue experiments indicated that circ_0001273 knockdown inhibited EC cell malignant phenotypes via increasing miR-622 expression.

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SLC1A5 was monitored in tumor tissues relative to normal tissues, and SLC1A5 expression was negatively correlated with miR-622 expression in tumor tissues (Figures 5d–f). SLC1A5 expression was also increased in TE1 and ECA109 cells compared with that in HEEC cells (Figure 5g). The data confirmed that SLC1A5 was a target of miR-622.

**SLC1A5 overexpression reversed the effects of miR-622 enrichment and promoted EC cell malignant phenotypes**

The expression of SLC1A5 was pronouncedly weakened in TE1 and ECA109 cells with miR-622 transfection, while its expression was largely recovered in cells with miR-622 + SLC1A5 transfection (Figure 6a). In function, miR-622 enrichment effectively weakened cell viability, the number of EdU-positive cells and the number of colonies, while SLC1A5 reintroduction recovered cell viability, the number of EdU-positive cells and the number of colonies (Figures 6b–d). MiR-622 upregulation-induced cell apoptosis was partially alleviated by SLC1A5 overexpression (Figure 6e). Cell migration distance repressed by miR-622 enrichment was largely facilitated by SLC1A5 reintroduction (Figure 6f). In addition, the enhanced E-cadherin expression and the impaired N-cadherin expression caused by miR-622 upregulation were largely reversed by SLC1A5 overexpression (Figure 6g,h). Moreover, glutamine consumption and glutamate production suppressed by miR-622 upregulation were remarkably recovered by additional SLC1A5 overexpression (Figure 6i,j). More importantly, the expression of SLC1A5 protein was markedly reduced in TE1 and ECA109 cells transfected with sh-circ_0001273 but partially restored in cells transfected with sh-circ_0001273 + anti-miR-622 (Figure 6k), suggesting that circ_0001273 positively modulated SLC1A5 expression via targeting miR-622.
Circ_0001273 downregulation repressed tumorigenesis in vivo by modulating miR-622 and SLC1A5 expression

ECA109 cells with downregulated circ_0001273 downregulation were subcutaneously injected into nude mice to induce tumorigenesis. As a result, circ_0001273 downregulation resulted in a notable decrease of tumor volume and tumor weight, thus repressing tumor growth (Figure 7a, b). Circ_0001273 expression was markedly decreased, while miR-622 expression was markedly reinforced in tumor tissues from the sh-circ_0001273 group (Figure 7c,d). The expression of SLC1A5 protein was also significantly reduced in tumor tissues from the sh-circ_0001273 group (Figure 7e). Further, IHC assay presented that the abundance of E-cadherin was reinforced, while the abundance of N-cadherin, Ki67 and SLC1A5 was decreased in tumor tissues from the sh-circ_0001273 group (Figure 7f). The findings showed that circ_0001273 downregulation inhibited tumorigenesis and tumor growth via downregulating SLC1A5 and upregulating miR-622.

DISCUSSION

The main findings in the current study were that circ_0001273 was aberrantly upregulated in EC, and inhibition of circ_0001273 repressed EC cell malignant behaviors and solid tumor growth. MiR-622 was ensured to be a target of circ_0001273, and miR-622 bound to SLC1A5 3’UTR to suppress SLC1A5 expression. Rescue experiments verified their interplays in EC cell development, uncovering that circ_0001273 controlled the miR-622/SLC1A5 pathway to regulate EC progression in vitro and in vivo.

Accumulating evidence suggests that circRNAs which are aberrantly regulated in cancer tend to mediate carcinogenesis or cancer suppression depending on their expression patterns in cancer. For example, both circRNA expression profiling and qPCR data illustrated the upregulation of circ_0006948 in ESCC, and circ_0006948 contributed to ESCC cell migration and invasion. In contrast, circ_SMAD7 was exposed to be downregulated in ESCC, and circ_SMAD7 ectopic expression sequestered ESCC cell colony formation and migration. We analyzed a circRNA expression profile from GEO database and focused on circ_0001273 that was highly expressed in plasma of ESCC patients. In view of the limitations of the current studies on circ_0001273, we aimed to address its functional effects on EC development. Interestingly, the upregulation of circ_0001273 was verified in both EC tissues and cell lines. Functional assays revealed that circ_0001273 knockdown restrained EC cell proliferation, migration and glutamine metabolism. The glutamine pathway is established to be an active metabolic pathway in the development of multiple cancers and is essential to maintain the fundamental functions of cancer cells. The target inhibition of glutamine metabolism has been proposed as a promising therapy for cancer treatment. Our data showed that circ_0001273 knockdown mediated glutamine metabolism inhibition, indicating that targeting circ_0001273 is of great significance for clinical treatment of EC, which needs further to be verified.

Regarding the regulatory mechanism of circ_0001273, we identified the downstream miRNAs targeted by circ_0001273 because circRNA may function as sponge of target miRNA to sequester miRNA expression. Both Circinteractome and CircBank predicted the binding between miR-622 and circ_0001273, which was further validated by dual-luciferase reporter experiment. It has universally been demonstrated that miR-622 expressed with a low level in tumor tissues, and miR-622 overexpression in cancer cells consistently inhibited cell proliferation, angiogenesis, migration and invasion. As well as in EC, miR-622 was poorly expressed in EC tissues and cells, and low miR-622 expression was related to poor survival rate and aggressive clinicopathological characteristics. In detailed function, miR-622 overexpression enhanced EC cell apoptosis and repressed cell growth, migration and invasion. The findings indicated the antitumor property of miR-622 in EC, and our results presented a consistent view. However, circ_0001273 sequestered miR-622 expression and thus exhibited cancer-promoting effects, suggesting that circ_0001273 knockdown-relieved miR-622 repression might be a strategy for EC therapy.

Among the predicted target genes of miR-622, SLC1A5 was of great interest because it has been found to play a key role in the transport of glutamine and essential amino acids in cancer metabolic reprogramming. For instance, SLC1A5 promoted glutamine transport required for lung cancer cell growth and motility. SLC1A5 high expression predicted poor overall survival and was linked to high stage and malignant characteristics of tumors. SLC1A5 showed high expression in EC tissues, and SLC1A5 downregulation in vitro induced EC cell cycle arrest, apoptosis and blocked leucine and glutamine transport. In addition, ESCC patients with high SLC1A5 expression were found to be prone to tumor metastasis and invasion after surgical resection, and SLC1A5 might be used as a prognostic biomarker. These findings highlighted the carcinogenic properties of SLC1A5 in common cancers. Our study reported that SLC1A5 served as a target of miR-622, and its overexpression restored EC cell proliferation, migration and glutamine metabolism that were blocked by miR-622. Moreover, circ_0001273 positively regulated SLC1A5 expression via targeting miR-622, indicating that circ_0001273 controlled the miR-622/SLC1A5 axis in EC progression.

In conclusion, the elevated expression of circ_0001273 was identified in EC tumor tissues and cell lines. Silencing circ_0001273 knockdown suppressed EC cell proliferation, survival, migration and glutamine metabolism, which was partially attributed to the regulation of miR-622 as well as downstream SLC1A5 by circ_0001273. Overall, these findings have provided new insights into the pathogenesis of EC.
CONFLICT OF INTEREST
The authors declare that they have no conflict of interests.

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