Transferrin receptor modulated by microRNA-497-5p suppresses cervical cancer cell malignant phenotypes

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A  – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Abstract

Background. Cervical cancer is prevalent throughout the world, and microRNA-497-5p (miR-497-5p) plays an important role in its development. However, the specific mechanism by which miR-497-5p targets the transferrin receptor (TFRC) during cervical cancer development has not been clarified.

Objectives. The aim of the study was to unravel TFRC expression and its role in cervical cancer cells, as well as the impact of the miR-497-5p/TFRC axis on cervical cancer cells.

Materials and methods. The target mRNA was determined through differential analysis, followed by the evaluation of its impact on survival and clinical staging. Then, quantitative real-time polymerase chain reaction (qPCR) was conducted to analyze the TFRC mRNA level in cervical cancer cells and normal cervical epithelial cells. Western blot (WB) was utilized to examine the expression levels of TFRC, cleaved caspase-3, cleaved caspase-9, and epithelial–mesenchymal transition (EMT)-related proteins. The miRNAs upstream of the target mRNA were predicted, and Pearson correlation analysis was performed, followed by the validation through the dual-luciferase reporter assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and colony formation assays were performed to analyze cancer cell viability, followed by a transwell assay aimed at measuring cell migratory and invasive abilities. Finally, flow cytometry was conducted to examine cell apoptosis and cell cycle.

Results. The transferrin receptor was significantly increased in cervical cancer cells and positively associated with clinical T and N stages. Silencing TFRC could constrain cell proliferative, migratory and invasive abilities, arrest the cell cycle and facilitate cell apoptosis in cervical cancer cells. The bioinformatics analysis showed a significantly negative correlation between miR-497-5p and TFRC in cervical cancer. Moreover, upregulated miR-497-5p hampered cervical cancer progression and decreased TFRC expression. The overexpression of TFRC reversed the suppressive impact of miR-497-5p overexpression on cervical cancer progression.

Conclusions. The modulatory role of the miR-497-5p/TFRC axis was confirmed in cervical cancer cells. This axis may present a new avenue for the diagnosis of cervical cancer and provide a novel target for cervical cancer treatment.

Key words: miR-497-5p, TFRC, biological function, cervical cancer
Background

Cervical cancer is a gynecological malignancy with increasing morbidity and mortality worldwide, especially in developing countries. In China, cervical cancer mortality ranks 4th among all cancers, and 2nd among cancers in women. Furthermore, it is commonly reported in patients aged 40–50 years old in China, and there are approx. 500,000 newly diagnosed cervical cancer cases each year. Cervical cancer may be induced by a variety of modalities and may develop complications during its progression, making its treatment complex and difficult. Although novel diagnostic and therapeutic technologies for cervical cancer are underpinned by extensive clinical research, leading to a 30–50% 5-year survival rate in patients at advanced stages, the therapeutic efficacy is still poor due to tumor recurrence and metastasis. Therefore, further investigation of effective targets for cervical cancer treatment is warranted.

The transferrin receptor (TFRC), namely CD71, participates in iron homeostasis and cell growth, and TFRC is often upregulated in tissue with a high proliferation index. The TFRC/CD71 is overexpressed in several human malignancies, such as lymphoma, liver cancer, colon cancer, and endometrial cancer, and may be associated with tumor stage and progression. The iron uptake by TFRC is an essential approach for cellular iron absorption, and TFRC is significantly dysregulated in many malignant cell types. However, the expression of TFRC in cervical cancer as well as its molecular mechanism are still not fully understood.

Aberrantly expressed microRNAs (miRNAs) are associated with tumorigenesis. Some miRNAs can act as oncogenes to promote tumorigenesis, while others serve as tumor suppressor genes. Numerous studies have demonstrated that miRNAs are a new class of tumor markers for cancer diagnosis, therapy and prognosis. A previous study unveiled that miRNAs repressed target gene expression by interfering with the genes or inhibiting mRNA translation. Accordingly, understanding the targets and regulatory mechanisms of miRNAs contributes to easier application of miRNAs in the cancer field. Recently, microRNA-497-5p (miR-497-5p) has been shown to be aberrantly expressed in several different cancers. For example, miR-497-5p modulates FGF2 to hamper proliferative and invasive properties of non-small cell lung cancer (NSCLC). Moreover, Zheng et al. proposed that miR-497-5p targeted HMGA2 to hinder hepatocellular carcinoma cell proliferation and metastasis. Furthermore, miR-497-5p/PDK3 restrains gastric cancer cell proliferation and cell cycle. However, the molecular mechanism of miR-497-5p in cervical cancer is lacking, and together with the lack of a targeted treatment represents an urgent research need.

Objectives

The aim of this study was to explore the mechanism of miR-497-5p and its ability to modulate TFRC in cervical cancer. The findings will contribute to the application of miR-497-5p/TFRC in cervical cancer diagnosis and prognosis.

Materials and methods

Bioinformatics methods

Expression profiles of mature miRNAs (normal: 3, tumor: 309) and mRNAs (normal: 3, tumor: 306) as well as clinical data from cervical cancer patients were obtained from The Cancer Genome Atlas (TCGA) database. The “edgeR” package was employed for a differential analysis of miRNAs and mRNAs between normal and tumor groups (|logFC| > 1.5, p < 0.05), allowing for the determination of differentially expressed miRNAs (DEmiRNAs) and mRNAs (DEmRNAs). The target mRNA was selected according to previous literature. Pearson correlation analysis was conducted to determine upstream miRNA, and upstream regulatory miRNAs of the target mRNA were identified using starBase, TargetScan and mirDIP databases. Finally, the survival analysis was carried out on the target mRNA by using the “survival” package in R. The relationship between the clinical stage and differential gene expression of miRNA was also analyzed.

Cell culture

Human normal cervical epithelial cell line HcerEpic (BNCC340374), and 4 human cervical cancer cell lines, namely Hela (BNCC337633), SiHa (BNCC102118), Caski (BNCC338223), and C33A (BNCC341097) were accessed from BeNa Culture Collection (Beijing, China). The HcerEpic, SiHa and C33A cells were cultured in Dulbecco’s modified Eagle medium (DMEM) (Gibco, Grand Island, USA) with 10% fetal bovine serum (FBS) (Gibco), while the other cervical cells were kept in RPMI-1640 medium with 10% FBS. Penicillin and streptomycin were added to all the media, and all cultures were maintained with 5% CO2 at 37°C.

Cell transfection

The sh-TFRC (TFRC knockdown treatment) and sh-NC (control of TFRC knockdown), as well as pcDNA3.1-constructed oe-TFRC (TFRC overexpression treatment) and oe-NC (control of TFRC overexpression) plasmids were procured from GeneChem (Shanghai, China). Lipo-fectamine™ RNAiMAX (cat. No. 13778150; Invitrogen, Waltham, USA) was applied to perform the cell transfection. A miR-497-5p mimic (miR-497-5p overexpression
treatment) and NC-mimic (control of miR-497-5p overexpression) (GenePharma, Suzhou, China) were transfected into cervical cancer cells with Lipofectamine™ 2000 (cat. No. 11668019; Invitrogen). Samples were cultured for 2 days in the corresponding medium at 37°C with 5% CO₂. After culture, transfection efficiency was detected using quantitative real-time polymerase chain reaction (qPCR).

**qPCR**

The miR-497-5p and TFRC mRNA expression levels in cervical cancer cells were assayed using qPCR. The TRIzol reagent (Thermo Fisher Scientific, Waltham, USA) was utilized for total RNA extraction, and the total RNA concentration was assessed with a NanoDrop ND-1000 instrument (NanoDrop Technologies, Wilmington, USA). Specific primers (Ribobio, Guangzhou, China) and prime script RT reagent kit (Takara, Kusatsu, Japan) were employed to reverse transcribe miRNA and mRNA into cDNA. Next, SYBR-Green PCR kit (Applied Biosystems, Waltham, USA) and SYBR® Premix Ex Taq™ II kit (Takara) were employed to quantify miRNA and mRNA expression, respectively. Finally, qRT-PCR was conducted on an ABI Prism 7500 fast real-time PCR system (Applied Biosystems, Waltham, USA) to quantify and compare relative expression levels of miR-497-5p or TFRC mRNA in control and experiment groups. Primer sequences are detailed in Table 1.

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<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Amplicon size</th>
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<td>miR-497-5p</td>
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<td></td>
<td>R: 5’-TGCTCAAGCAGCAGACTG-3’</td>
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<td></td>
<td>R: 5’-CCTGGAAGATCGTGATGGGATT-3’</td>
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**Western blot**

Cervical cancer cells in different transfection groups were lysed on ice for 30 min using a pre-cooled radiomunoprecipitation reagent (Beyotime, Shanghai, China). The supernatant was removed and centrifuged at 14,000 rpm and 4°C, followed by collection. Total proteins were determined with a BCA protein concentration kit (RTP7102; Real-Times Biotechnology, Beijing, China). Next, protein samples (20 µg) were separated on a 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Corning Inc., Corning, USA). Then, the membranes were blocked with 5% skimmed milk at room temperature for 2 h, and probed with monoclonal antibodies: rabbit anti-TFRC (ab214039, 1:1000; Abcam, Cambridge, UK), rabbit anti-GAPDH (ab181602, 1:10000; Abcam), rabbit anti-cleaved caspase-3 (ab32042, 1:500; Abcam), rabbit anti-cleaved caspase-9 (SAB4503337, 1:500; Merck, Rahway, USA), rabbit anti-E-cadherin (ab40772, 1:5000; Abcam), rabbit anti-N-cadherin (ab76011, 1:5000; Abcam), and rabbit anti-vimentin (ab92547, 1:5000; Abcam) overnight at 4°C. Membranes were rinsed 3 times (15 min/time) with phosphate-buffered saline (PBS) and Tween-20, after which they were cultured with goat anti-rabbit IgG H&L (HRP) (ab6721, 1:2000; Abcam) at room temperature for 1 h. Afterward, images were acquired using a chemiluminescence detection kit (Sigma-Aldrich, St. Louis, USA) in the darkroom. ImageJ software (National Institutes of Health, Bethesda, USA) was used for statistical analysis of the results.

**MTT and colony formation assays**

Cells were cultured in 96-well plates (5×10³ cells/well), and after transfection for 0, 24, 48, 72, and 96 h, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 g/L; Sigma-Aldrich) was added for another 4 h of cell incubation at 37°C. Then, 150 µL of dimethyl sulfoxide (DMSO) was supplemented to each well to dissolve crystal violet. Finally, a spectrophotometer was utilized to detect the absorbance at 570 nm.

The 6-well plates (5×10⁵ cells/well) were recommended for cell seeding, and the culture medium was replaced every 3 days. After 10 days, the cells were rinsed twice with 1×PBS, followed by 20 min of 4% paraformaldehyde and 5 min of crystal violet. Five fields were selected under an optical microscope (model CKX53; Olympus Corp., Tokyo, Japan). The number of colonies was counted.

**Cell migration and invasion assays**

Mitomycin C (MedChemExpress, Monmouth Junction, USA) (10 µg/mL) was used to eliminate the influence of cell proliferation. The 24-well insert and polycarbonate membrane with 8.0-µm well (Millipore, Burlington, USA) (10 µg/mL) was used to eliminate the influence of cell migration. The 6-well plates were recommended for cell seeding, and the culture medium was replaced every 2 days. After 10 days, the cells were rinsed twice with 1×PBS, followed by 20 min of 4% paraformaldehyde and 5 min of crystal violet. Five fields were selected under an optical microscope (model CKX53; Olympus Corp., Tokyo, Japan). The number of colonies was counted.

**Table 1.** Primer sequences in quantitative real-time polymerase chain reaction (qPCR)

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Cell apoptosis and cell cycle analysis

Apoptosis was analyzed using a cell apoptosis kit (BD Biosciences) according to the manufacturer’s instructions. First, the cells were prepared into a single-cell suspension, which was re-suspended with PBS after centrifugation. Then, the negative and positive control groups were prepared. For the assessment of cell apoptosis, propidium iodide (PI) and Annexin V were used to stain the cells, followed by flow cytometry using a FACSCanto™ II (BD Biosciences). To analyze the cell cycle, staining was conducted with PI only, followed by flow cytometry.

Dual-luciferase reporter gene assay

First, gene sequences of the 3’-untranslated region (3’-UTR) of wild-type (WT) or mutant (MUT) TFRC were cloned into pmir-GLO vector (Promega, Madison, USA), and named TFRC-WT and TFRC-MUT, respectively. The miR-497-5p mimic/NC mimic and TFRC-WT/TFRC-MUT vectors were transfected into cervical cancer cells with the use of Lipofectamine™ 2000 (Invitrogen). Then, luciferase activity was evaluated using a dual-luciferase reporter detection system (Promega).

Statistical analyses

All collected data were processed using GraphPad Prism v. 6.0 (GraphPad Software, San Diego, USA), while the “edgeR” package and “survival” package were processed with R software v. 4.0.2 (MathSoft, Cambridge, USA). All in vitro experiments were repeated 9 times. Due to the limited statistical sample size, which was insufficient for normal distribution, the data did not meet the assumption of normal distribution. Therefore, Mann–Whitney U tests were used to make the comparison between the 2 groups, a Kruskal–Wallis test was used to test the comparison between more than 2 groups, and Dunn’s test was applied to conduct survival analysis, and Pearson correlation analysis was utilized to determine upstream miRNAs of the target gene. All data are expressed as mean ± standard deviation (M ±SD), with p < 0.05 representing statistical significance, p < 0.01 indicating a significant difference and p < 0.001 denoting an extremely significant difference.

Results

TFRC expression is significantly increased in cervical cancer cells

To identify the differentially expressed genes in cervical cancer, mRNAs in the TCGA database were categorized, with |logFC| > 1.5 and false discovery rate (FDR) <0.05 as cutoff standards. Through the differential expression analysis conducted using the “edgeR” package, a total of 2956 DEmRNAs were obtained, among which 1274 genes were upregulated (red dots), 1682 genes were differentially downregulated (green dots), and the black dots represented genes with insignificant differences (Fig. 1A). Although previous studies have reported that concomitant upregulation of TFRC in various tumor tissues is associated with poor prognosis and facilitates cancer cell proliferation and metastasis,9,30 its role in cervical cancer is less understood. Therefore, TFRC was selected for follow-up investigations. The results of the differential expression analysis, as presented in Fig. 1B, revealed an increased TFRC expression in cervical cancer tissue compared to normal tissue (p < 0.05, Mann–Whitney U test). Meanwhile, clinical data demonstrated that TFRC expression displayed a significant difference in various clinical T/N stages with an increasing trend (p < 0.01, Kruskal–Wallis test; p < 0.05, Mann–Whitney U test) (Fig. 1C,D). The survival analysis further showed that the upregulation of TFRC in cervical cancer tissue influenced a patient’s prognosis (p < 0.050, Mann–Whitney U test) (Fig. 1E), with the survival time of patients with decreased TFRC level being longer than those with increased TFRC expression. Relative to HcerEpic cells, TFRC expression was increased in Hela, SiHa, Caski, and C33A cells (Hela compared to HcerEpic: p < 0.001, SiHa compared to HcerEpic: p < 0.001, Caski compared to HcerEpic: p < 0.01, C33A compared to HcerEpic: p < 0.05, Kruskal–Wallis test with Dunn’s post hoc test) (Fig. 1F). Therefore, Hela and SiHa cell lines with relatively high TFRC levels were chosen for further functional experiments.

TFRC silencing contributes to attenuating cancer cell malignant phenotypes

To identify the impact of TFRC on cancer cells, sh-TFRC and sh-NC were transfected into Hela and SiHa cell lines. Transfection efficacy was verified using qPCR, showing that the TFRC level was significantly decreased in the sh-TFRC group (p < 0.05, Mann–Whitney U test) (Fig. 2A). To investigate whether TFRC expression affects cervical cell behaviors and phenotypes, several cell function experiments were introduced. Silencing TFRC restrained cervical cancer cell proliferation (p < 0.05, Mann–Whitney U test) (Fig. 2B), while colony formation assays demonstrated that silencing TFRC resulted in fewer colonies of Hela and SiHa cells relative to the sh-NC group (p < 0.05, Mann–Whitney U test) (Fig. 2C). To define the impact of TFRC expression on cell migration and invasion, a transwell assay was performed, showing that cell migration and invasion were significantly reduced in the sh-TFRC group compared to those in the sh-NC group (p < 0.05, Mann–Whitney U test) (Fig. 2D,E). Furthermore, the cell apoptosis assay illustrated that silencing TFRC significantly upregulated (p < 0.05, Mann–Whitney U test) the cell apoptosis rate (Fig. 2F). Considering the influence of TFRC on the cell cycle, flow cytometry was used to examine the cell cycle
Inhibiting the expression of TFRC could arrest the cell cycle in cervical cancer cells in the G0/G1 phase (p < 0.05, Mann–Whitney U test) (Fig. 2). These findings highlighted that silencing TFRC hampered the progression of cervical cancer cell phenotype in vitro.

**miR-497-5p inhibits the TFRC expression in cervical cancer cells**

To screen potential upstream regulatory miRNAs for TFRC, miRNAs in the TCGA database were categorized, with |logFC| > 1.5 and FDR < 0.05 set as cutoff standards. A differential analysis was conducted using the "edgeR" package, highlighting 128 DEmiRNAs, among which 76 genes were upregulated (red dots), 52 genes were differentially downregulated (green dots), and the black dots represented miRNAs with insignificant differences (Fig. 3A). Next, the upstream regulatory miRNAs of TFRC were mined using bioinformatics databases. Predicted miRNAs overlapped with downregulated DEmiRNAs, and thus we obtained 8 DEmiRNAs with binding sites into TFRC (Fig. 3B). Then, the correlation analysis was conducted on TFRC and 8 DEmiRNAs, and a negative correlation was found between miR-497-5p and TFRC, along with the highest coefficient (p = −0.27) (Fig. 3C). Through bioinformatics analysis, we theorized that miR-497-5p level was lowered in cervical cancer tissue compared to normal tissue (p < 0.001, Mann–Whitney U test) (Fig. 3D). Therefore, miR-497-5p was selected for further analysis.

A binding site analysis demonstrated that miR-497-5p was a direct binding partner of TFRC (Fig. 3E). To investigate whether miR-497-5p targets TFRC by binding to its 3’UTR, WT or MUT TFRC luciferase reporter plasmid and miR-497-5p mimic or NC mimic were co-transfected into Hela and SiHa cells. The luciferase activity assay showed that luciferase activity of TFRC-WT in Hela and SiHa cell lines in the miR-497-5p mimic group was significantly reduced (p < 0.05, Mann–Whitney U test), and no substantial difference was observed in the TFRC-MUT group (p > 0.05, Mann–Whitney U test) (Fig. 3F). Furthermore, the upregulation of miR-497-5p significantly reduced TFRC mRNA (p < 0.05, Mann–Whitney U test) and protein levels, as assessed through qPCR and WB (Fig. 3G,H). Hence, it could be determined that miR-497-5p inhibited TFRC expression.

To investigate the influence of miR-497-5p on cervical cancer cells, a miR-497-5p overexpression cell line was established for cell function experiments. Western blot (WB) analysis revealed that when miR-497-5p was overexpressed, protein expression of apoptosis-related proteins, namely cleaved caspase-3 and cleaved caspase-9, was significantly elevated compared with the NC group.
X. Fang et al. MiR-497-5p/TFRC axis affects cervical cancer (Supplementary Fig. 1). The transwell assay further demonstrated that the overexpression of miR-497-5p inhibited cervical cell migration and invasion compared with the NC group (p < 0.05, Mann–Whitney U test) (Supplementary Fig. 2). Next, we detected levels of epithelial–mesenchymal transition (EMT)-related proteins using WB, showing that the overexpression of miR-497-5p hampered N-cadherin and vimentin protein levels, and fostered E-cadherin protein levels compared with the NC group (p < 0.050, Mann–Whitney U test) (Supplementary Fig. 3). These results illustrated that miR-497-5p could hamper migration and invasion of cervical cancer cells, but foster cell apoptosis.

Fig. 2. Silencing transferrin receptor (TFRC) hinders cervical cancer cell phenotype progression. A. Transfection efficacy in Hela and SiHa cells (Mann–Whitney U test); B. Proliferative property of cervical cancer cells (Mann–Whitney U test); C. Cell colony formation of cervical cancer cells (Mann–Whitney U test); D,E. Cell migration and invasion in various treatment groups (100×) (Mann–Whitney U test); F. Apoptosis rate of cervical cancer cells in varying transfection groups (Mann–Whitney test); G. Cell cycle in differently transfected cell lines (Mann–Whitney U test)

*p < 0.05 indicated statistically significant values; OD – optical density.
miR-497-5p hampers malignant progression of cervical cancer cells by downregulating TFRC

To determine whether miR-497-5p modulates the development of cervical cancer cells via TFRC, a miR-497-5p mimic was utilized to upregulate miR-497-5p. Simultaneously, the miR-497-5p mimic and oe-TFRC were co-transfected into cervical cancer cells. Through qRT-PCR and WB, it was observed that the upregulation of miR-497-5p decreased TFRC expression compared to the NC mimic+oe-NC group, and TFRC level was elevated in the miR-497-5p mimic+oe-TFRC group compared with the NC group (p < 0.05, Mann–Whitney U test) (Fig. 4A,B). The MTT and colony formation assay unveiled that proliferation and colony formation of Hela and SiHa cells were hindered by transfecting the miR-497-5p mimic, but the inhibitory impact was partially eliminated by co-transfecting with oe-TFRC (p < 0.05, Mann–Whitney U test) (Fig. 4C,D). Moreover, migratory and invasive properties were weakened in cervical cancer cells that overexpressed miR-497-5p compared with the NC group, whereas TFRC overexpression reversed this inhibitory effect and returned to NC group levels (p < 0.05, Mann–Whitney U test) (Fig. 4E,F). Moreover, flow cytometry showed that increased miR-497-5p resulted in increased apoptosis of Hela and SiHa cells compared with the NC group, but simultaneous overexpression of miR-497-5p and TFRC reduced the apoptosis (p < 0.05, Mann–Whitney U test) (Fig. 4G). Similarly, miR-497-5p facilitated cell cycle arrest in the G0/G1 phase, but the cell cycle was restored in the NC group by additional transfection of oe-TFRC (p < 0.05, Mann–Whitney U test) (Fig. 4H). Together, these findings revealed that TFRC modulated cervical cancer cell progression via the restraining influence of miR-497-5p.

Discussion

Patients with advanced cervical adenocarcinoma are largely incurable due to local recurrence and metastatic diffusion. As reported previously, many mRNAs are...
MiR-497-5p/TFRC axis affects cervical cancer and is implicated in tumorigenesis and progression. Of note, exploring the potential mechanism of cervical cancer may lead to its early diagnosis and effective therapy. Transferrin receptor is present in almost all mammalian cells. Herein, TFRC expression data in cervical cancer were analyzed for the mechanism of TFRC dysregulation in the pathogenesis of the disease, finding that TFRC was upregulated, and silencing TFRC repressed the progression of cervical cancer cell phenotype. Previous studies have uncovered that TFRC is increased in both colon and lung cancers. Specifically, Fu et al. highlighted that TFR1 was significantly increased and was essential in the malignant progression of colorectal cancer. Furthermore, Whitney et al. confirmed that...

Fig. 4. MicroRNA-497-5p (miR-497-5p) represses cell phenotype progression in cervical cancer through transferrin receptor (TFRC) downregulation. A-B. TFRC mRNA and protein levels in cervical cancer cells (Hela and SiHa) (Mann–Whitney U test); C. Cell proliferation in varying groups (Mann–Whitney U test); D. Colony formation of cervical cancer cells in various treatment groups (Mann–Whitney U test); E-F. Cell migration and invasion in various treatment groups (100×) (Mann–Whitney U test); G. Cell apoptosis in various treatment groups (Mann–Whitney U test); H. Cell cycle statuses in various groups (Mann–Whitney U test) *p < 0.05 indicated statistically significant values; OD – optical density.
TFRC was significantly increased in NSCLC.\textsuperscript{36} Our work is concordant with an earlier report that compared the up-regulation of TFRC in cervical cancers to adjacent non-tumor tissue and correlated these results to progression stages, tumor status and lymph node involvement.\textsuperscript{27} These findings show that TFRC may function as a direct and indirect target for the administration of therapeutic agents, presenting a potential prospect in treating cancer cell malignant progression.

This work uncovered the mechanism of miR-497-5p modulating TFRC in cervical cancer cells. Earlier studies have shown that miR-497-5p, miR-320 and miR-210 target TFRC.\textsuperscript{38,39} Herein, we have demonstrated that TFRC was a direct target of miR-497-5p. Moreover, through bioinformatics analysis, a negative link between miR-497-5p and TFRC was uncovered. Our finding is congruous with previous evidence stating that the expression of miR-497-5p is reduced in gastric cancer,\textsuperscript{26} while Li et al. proposed that miR-497-5p is significantly decreased in NSCLC.\textsuperscript{30} Most importantly, increased miR-497-5p expression decreased TFRC in cervical cancer cells, while miR-497-5p and TFRC overexpression restored the repressive influence of overexpressing miR-497-5p on cervical cancer. This result was also supported by Chen et al.\textsuperscript{41} Given that miR-497-5p targets TFRC to repress cervical cancer tumorogenesis and progression, it may represent a feasible therapeutic target for cervical cancer patients.

Limitations

While the findings of the study are promising, an analysis of these aspects in animal models and clinical trials would be valuable. Therefore, future research will focus on the mechanism of the miR-497-5p/TFRC axis in cervical cancer from multiple perspectives in order to offer a theoretical foundation for potential therapies.

Conclusions

Transferrin receptor knockdown could inhibit the proliferation, migration and invasion of cervical cancer cells while promoting apoptosis. Regarding the regulatory mechanism, concomitant upregulation of TFRC is caused by decreased miR-497-5p level, while the upregulation of miR-497-5p repressed the malignant phenotype of cervical cancer by modulating TFRC. This work has contributed to proposing more therapeutic regimens for cervical cancer.

Supplementary data

The supplementary materials are available at https://doi.org/10.5281/zenodo.8032200. The package contains the following files:

Supplementary Fig. 2. Cell migration and invasion abilities evaluated using transwell assay (Mann–Whitney U test).

Supplementary Fig. 3. Changes in expression of EMT-related proteins evaluated using WB (Mann–Whitney U test; *p < 0.050 represents statistically significant values).

References


