Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

MiR-214-3p suppresses cervical cancer cell metastasis by downregulating THBS2

Xu Wang[1,2], Jing Xu[2], Fang Hua[2], Yujing Wang[2], Guiying Fang[2], Hongzhen Zhang[2], Xiaohua Wu[1,3*]

[1] Hebei Medical University, Shijiazhuang, China
[2] Department of Obstetrics and Gynecology, the Frist Hospital of Hebei Medical University, Shijiazhuang, China
[3] Department of reproductive medicine, Shijiazhuang Fourth Hospital, Obstetrics and Gynecology Hospital Affiliated to Hebei Medical University, Shijiazhuang, China

ARTICLE INFO

Original paper

Article history:
Received: May 04, 2023
Accepted: June 25, 2023
Published: September 30, 2023

Keywords:

MicroRNA-214-3p, THBS2, cell invasion, cell migration, cervical cancer

ABSTRACT

Cervical cancer (CC) is the most common human gynecological malignancy worldwide. Recently, accumulating evidences revealed the critical functions of miRNAs in the occurrence and development of cervical cancer. In our study, we aimed to demonstrate the function of miR-214-3p in regulating cell metastasis in cervical cancer. We showed low expression of miR-214-3p in cervical cancer cells and demonstrated downregulation of miR-214-3p promoted cervical cancer metastasis. Furthermore, THBS2 was identified as a novel target of miR-214-3p in cervical cancer cells. miR-214-3p suppressed THBS2 expression by directly targeting 3'UTR of THBS2, resulting in the inhibition of cell viability, invasion and migration. Taken together, the results implied inhibited effects of miR-214-3p on cervical cancer and provided new insight into potential ways for cervical cancer diagnosis and treatment.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.9.30

Introduction

Cervical carcinoma, a highly prevalent gynecological malignancy, poses a significant threat to women's health worldwide (1). With over 500,000 new cases diagnosed each year and a high mortality rate (2), cervical cancer remains a major global health concern. While advancements in diagnostic and treatment techniques have contributed to a decline in the incidence and mortality of cervical cancer, its complex molecular regulation at the transcriptional and post-transcriptional levels continues to present challenges (3, 4).

In recent years, noncoding RNAs, particularly microRNAs (miRNAs/miRs), have emerged as a novel area of research that holds great promise for cancer diagnosis and understanding the underlying mechanisms of pathogenesis. MiRNAs are short, single-stranded noncoding RNAs approximately 22 nucleotides in length that function as post-transcriptional regulators by binding to specific mRNA targets. Numerous studies have implicated miRNAs in the development of cervical cancer, highlighting their roles in regulating cancer cell migration, invasion, epithelial-mesenchymal transition (EMT), and apoptosis (5-7).

One such miRNA of interest is miR-214, which has been implicated in various types of cancer and has shown both oncogenic and tumor-suppressive properties depending on the context. In human ovarian cancer, miR-214 downregulates PTEN levels by targeting its 3' untranslated region (3'UTR), leading to increased cell survival in the presence of cisplatin. Conversely, the downregulation of miR-214 results in PTEN accumulation and cell cycle arrest in the G1 phase in gastric cancer (8, 9). In breast cancer, miR-214 induces tumorigenesis by promoting the accumulation of Ezh2, thereby enhancing cell proliferation and invasion (10). Furthermore, miR-214 contributes to melanoma tumor progression through the suppression of TFAP2C (11).

In our study, we focused on miR-214-3p, a specific isoform of miR-214, and its potential involvement in cervical cancer. We observed downregulation of miR-214-3p in cervical cancer cells compared to normal human cervical cells. Through subsequent investigations, we identified thrombospondin-2 (THBS2) as a novel target of miR-214-3p. THBS2 is a multifunctional glycoprotein belonging to the thrombospondin (THBS) family and has been implicated in angiogenesis, apoptosis, cell adhesion, and cell motility through interactions with cell surface receptors, extracellular matrix proteins, and growth factors (12, 13). While previous studies have established THBS2 as an oncogene involved in the progression of melanoma, lung, and prostate tumors (14-19), limited evidence exists regarding its role in human cervical cancer (20-22).

Our study not only provides valuable insights into the dysregulation of miR-214-3p in cervical cancer but also uncovers its novel target, THBS2. By targeting the 3'UTR of THBS2, miR-214-3p effectively downregulates THBS2 expression in cervical cancer cells, leading to significant inhibition of cell viability, invasion, and migration. These findings collectively highlight the crucial role of miR-214-

* Corresponding author. Email: xiaohuawu65@126.com

3p in regulating cervical cancer metastasis.

The identification of miR-214-3p as a key player in cervical cancer metastasis adds to our understanding of the complex molecular mechanisms underlying this disease. Furthermore, our study offers new insights into potential diagnostic and therapeutic strategies for cervical cancer. By exploring the dysregulation of miRNAs, such as miR-214-3p, and their target genes, like THBS2, we can pave.

Materials and Methods

Cell culture and cell transfection

C33A, HEK293T cells were maintained in Dulbecco's modified eagle medium (DMEM) (Gibco; Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco; Rockville, MD, USA) and penicillin/streptomycin (100 u/ml) at 37°C and 5% CO_2. Human primary cervical epithelial cells (HUM-iCell-F016) were purchased from iCell Bioscience Inc (Shanghai, China) and maintained in a human primary epithelial cell medium (PriMed-iCell-001, iCell Bioscience Inc., Shanghai, China). miR-214-3p mimics (100 nM) and control were transfected into cells by using LipofectamineTM 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturers' instructions. The sequences of mimics and control: miR-214-3p mimics Sense 5′-ACAGCAGGCACAGACAGGCAGU-3′, Antisense 5′-UGCCUGUCUGUGCCUGCUGUUU-3′; miRNA NC Sense 5′-UUCUCCGAACGUGUCACGUTT-3′, Antisense 5′-ACGUGACACGUUCGGAGAATT-3′.

RNA extraction and qPCR

Total RNA was isolated using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed to cDNA with an RT kit (TaKaRa Biotechnology, Tokyo, Japan). miR-214 -3p and THBS2 mRNA levels were measured by using real-time PCR. U6 and glyceraldheyde 3-phosphate dehydrogenase (GAPDH) were used as internal controls. Primer sequences used for the reactions were as follows: THBS2 (sense, 5'-GCGGCTGGGTCTATTTGTC-3', antisense, 5'-GCAGGAGGTGAAGAACCATC-3'), GAPDH (sense, 5'-CTCTCTGCTCCTCCTGTTCGAC-3', antisense, 5'-TGAGCGATGTGGCTCGGCT-3'), miR-214-3p (sense, 5'-CTCTCTGCTCCTCCTGTTCGAC-3', antisense, 5'-TGAGCGATGTGGCTCGGCT-3'), U6 (sense, 5'-TGAGCGATGTGGCTCGGCT-3', antisense, 5'-GTATCCAGTGCGTGTCGTGG-3').

Western blotting

Total proteins were extracted and determined using a bicinchoninic acid kit (P0010; Beyotime Institute of Biotechnology, Shanghai, China). Proteins (50 μg) were separated using 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After blocking with 5% non-fat milk at 37°C for 1 h, membranes were incubated with primary antibodies overnight at 4°C and HRP-conjugated secondary antibodies for 1 h at room temperature. Antibodies used were as followed: Anti-Thrombospondin 2 antibody (ab84469, Abcam, Cambridge, MA, USA), Anti-MMP2 antibody (ab92536, Abcam, Cambridge, MA, USA), Anti-MMP9 antibody (ab76003, Abcam, Cambridge, MA, USA), Anti-beta Actin antibody (ab8227, Abcam, Cambridge, MA, USA).

Cell viability

Cell viability was analysed by using a cell counting kit-8 (CCK-8) assay (Beyotime, Shanghai, China). In brief, cells were transfected with control and miR-214-3p mimics. 24 h after transfection, cells were re-seeded in a 96-well plate at a density of 1×10^5 cells/well to maintain 48 h. Cells were incubated with CCK-8 solution at 37°C for 2 h and measured with a microplate absorbance reader (Thermo Fisher, Waltham, MA, USA) at a wavelength of 450 nm.

Luciferase reporter assay.

Wide type THBS2 3' UTR (WT) and mutant THBS2 3' UTR (Mut) were generated into pGL4.10 vector respectively (Promega Corporation, Madison, WI, USA). HEK293T cells were transfected with miR-214-3p mimics or control mimics together with WT THBS2 3' UTR or mutant THBS2 3' UTR using LipofectamineTM 2000 reagent (Invitrogen). Cells were lysed and luciferase activity was detected using a dual luciferase reporter assay kit (Promega Corporation, Madison, WI, USA) 48h after transfection.

Scratched assay

Cells were seeded in a 6-well plate at a density of 1×10^5 cells/well. A scratch was performed using a sterile 200 μl pipette tip and cells were washed with PBS to remove detached cells. Images were taken at 0 h and 24 h after scratching by using a microscope.

Transwell migration assay

Transwell chambers were coated by Matrigel and rehydrated with serum-free DMEM in advance. Cells transfected with miRNAs as indicated were diluted and added to the upper chamber. DMEM with 10% FBS was added in the low chamber. After incubation for 6 h at 37°C, cells on the membranes were fixed and stained. Four random visual fields were counted for each sample.

Statistical analysis

All data were analysed using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA). Student's t-test was used for comparing two groups. For comparing multiple groups, one-way analysis of variance (ANOVA) or two-way ANOVA was used. Results were expressed as mean ± SEM. *, $P\leq0.05$; **, $P\leq0.01$; ***, $P\leq0.001$; ns, not significant.

Results

Cervical cancer cells have low miR-214-3p expression.

To determine the function of miR-214-3p in cervical cancer tumorigenicity, we analysed miR-214-3p levels both in human primary cervical epithelial cells (Control) and cervical cancer cells (C33A). Real-time PCR was performed to detect expression levels of miR-214-3p. As Figure 1 showed, cervical cancer cells exhibited a decreased expression of miRNA-214 compared with human normal cervical cells.

miR-214-3p directly targets THBS2 and downregulates THBS2 expression.

To determine miR-214-3p target genes involved in cervical cancer, we employed two bioinformatic algorithms

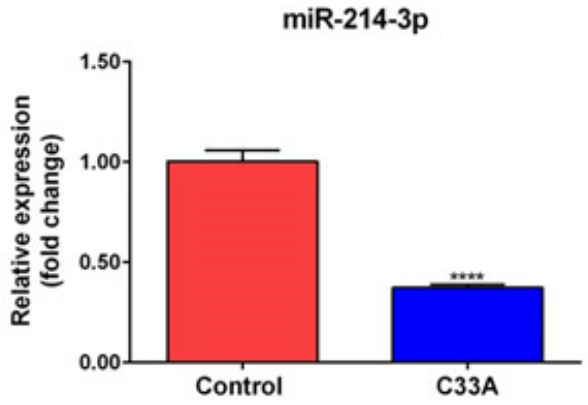

Figure 1. Expressions of miR-214-3p and THBS2 in normal cervical epithelial cells and cervical cancer cells. Expression of miR-214-3p was significantly lower in cervical cancer cells (C33A) than that in normal cervical epithelial cells (Control). ****, P≤0.001.

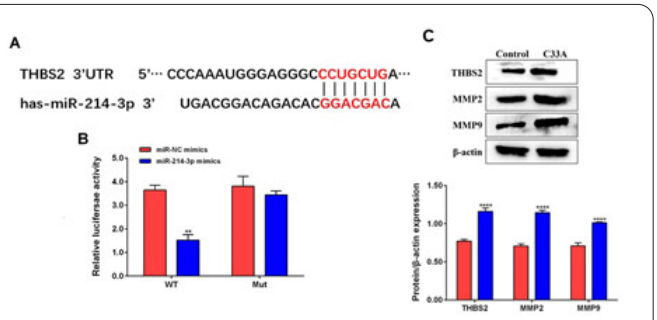

Figure 2. miR-214-3p directly targeted THBS2 in cervical cancer cells. A, The sequences of the predicted miR-214-3p binding sites on THBS2. B, miR-214-3p mimics were co-transfected to HEK293T cells with THBS2 WT and THBS2 Mut respectively to HEK293T cells. Cells were collected for luciferase assay 48h after transfection. C, Expression levels of THBS2, MMP2 and MMP9 were analysed and qualified. β-actin was used as a loading control. **, P≤0.01; ****, P≤0.001.

to predict the potential target candidates of miR-214-3p. Based on the prediction results, we found that THBS2 binds with miR-214-3p at 3′UTR of the gene (Figure 2A). For further confirmation, we performed a dual-luciferase reporter assay by evaluating the relative luciferase activities in HEK293T cells. We co-transfected reporter plasmid expressing the THBS2 3′UTR (WT) or THBS2 mutant 3'UTR (Mut) with miR-214 mimics. Dual-luciferase reporter assays demonstrated lower luciferase activities when we introduced mutation in THBS2 3′UTR (Figure 2B).

Next, we examined the protein levels of THBS2. We detected a high expression level of THBS2 in C33A compared with that in normal primary cervical cells (Figure 2C). In our study, western blotting also showed decreasing protein levels of MMP9 and MMP2 which are two key proteins that play critical functions in cancer cell metastasis regulated by THBS2 (22). The high expression level of THBS2 was greatly eliminated by overexpressing miR-214-3p mimics in C33A. Expressing levels of MMP9 and MMP2 were also reduced when miR-214-3p was overexpressed (Figure 3A). Meanwhile, we co-expressed exogenous THBS2 with miR-214-3p mimics to confirm the impact of miR-214-3p on THBS2 (Figure 3A). We found that overexpressing miR-214-3p mimics also significantly decreased exogenous THBS2 as shown in Figure 3A.

Taken together, these data indicated a direct interaction between miR-214-3p and the THBS2 3′UTR, which allows miR-214-3p to down-regulate THBS2 expression.

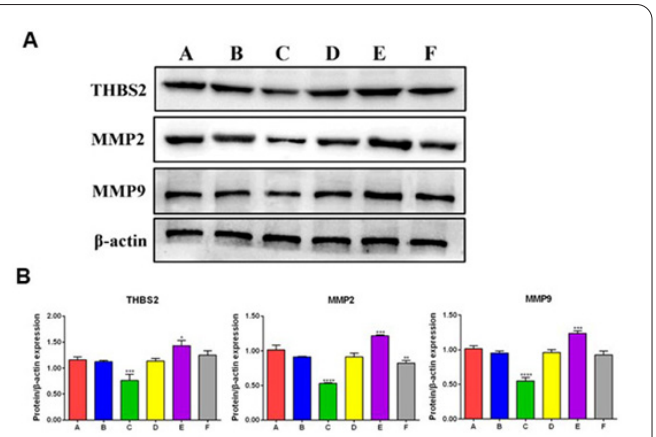

Figure 3. miR-214-3p suppressed protein levels of THBS2, MMP2 and MMP9 in C33A cells. (A) Expression levels of THBS2, MMP2 and MMP9 were analysed by Western blotting. β-actin was used as a loading control. (B) Western blotting qualification of THBS2, MMP2 and MMP9. A: Control C33A; B: C33A transfected with NC mimics; C: C33A transfected with miR-214-3p mimics; D: C33A overexpressing control plasmid; E: C33A overexpressing THBS2; F: C33A overexpressing miR-214-3p mimics and THBS2. **, p≤0.01; ****, p≤0.001.

miR-214-3p inhibits viability, migration and invasion of cervical cancer cells

THBS2 was reported to promote cancer metastasis. Based on the previous studies and the above results, we next determined the role of miR-214-3p in regulating cervical cell viability, migration and invasion. As expected, miR-214-3p impaired C33A cell viability as that shown in Figure 4. Whereas the elimination of cell viability was obviously recovered with overexpression of THBS2. To confirm the result, we overexpressed THBS2 in C33A to get higher cell viability. However, elevated cell viability was also remarkably inhibited in the presence of miR-214-3p (Figure 4).

To study the effects of miR-214-3p on cervical cancer

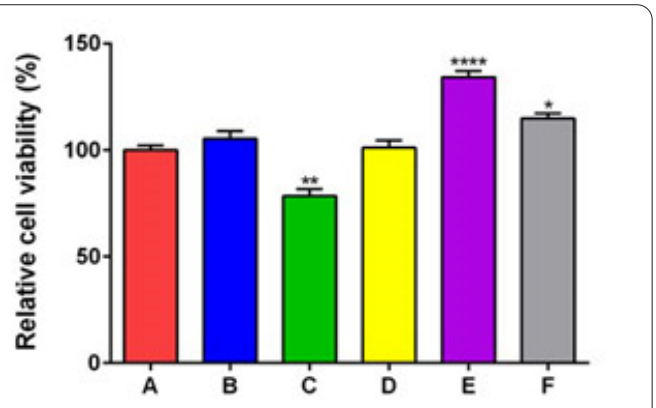

Figure 4. miR-214-3p significantly decreased cervical cancer cell viability. CCk-8 was used to analyse cell viability in C33A cells. A: Control C33A; B: C33A transfected with NC mimics; C: C33A transfected with miR-214-3p mimics; D: C33A overexpressing control plasmid; E: C33A overexpressing THBS2; F: C33A overexpressing miR-214-3p mimics and THBS2. *, P≤0.05; **, P≤0.01; ****, P≤0.001.

cell migration and invasion, scratch wound-healing assay and transwell assay were performed in our study. C33A cells were transfected with miR-214-3p mimics only or co-transfected with miR-214-3p mimics and a plasmid carrying THBS2. Cells were applied to a scratch wound-healing assay. The results demonstrated that miR-214-3p inhibited the migration of C33A cells across the scratch wound even in cells overexpressing THBS2 (Figure 5). Meanwhile, C33A expressing miR-214-3p mimics exhibited a decreased invasion ability compared with control cells. In addition, the accelerated cell invasion caused by THBS2 overexpression was also abrogated in presence of miR-214-3p (Figure 6).

All of the above results suggested that miR-214-3p suppresses cervical cancer cell viability, invasion and migration. And the inhibition that miR-214-3p performed on cell viability, invasion and migration was based on its downregulation of THBS2.

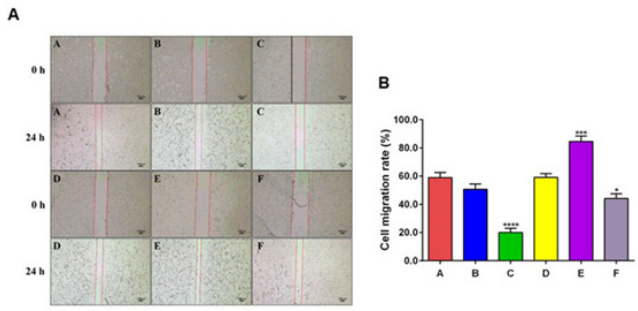

Figure 5. The closure of scratch wounds of C33A cells was inhibited by miR-214-3p. Wound-healing assay was used for detecting cell migration in C33A cells. A: Control C33A; B: C33A transfected with NC mimics; C: C33A transfected with miR-214-3p mimics; D: C33A overexpressing control plasmid; E: C33A overexpressing THBS2; F: C33A overexpressing miR-214-3p mimics and THBS2. ***, P≤0.01; ****, P≤0.001.

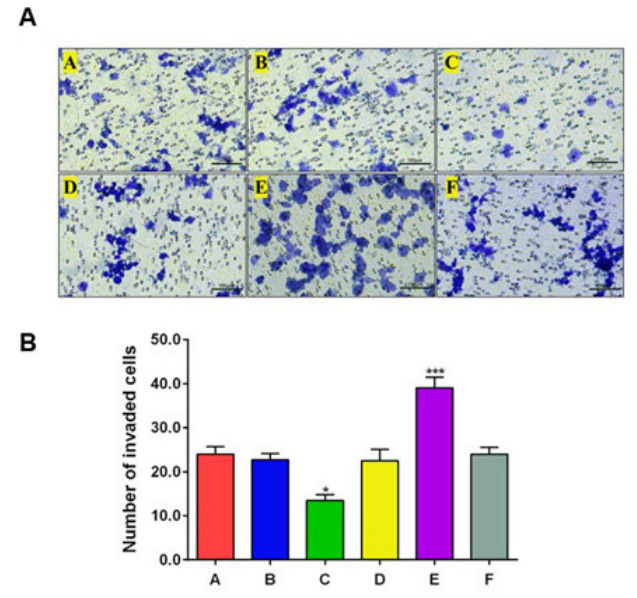

Figure 6. miR-214-3p impaired cervical cancer cell invasion ability. (A) Transwell assays were performed in C33A cells 24h post-transfection. (B) Transwell assays were counted under a microscope in four randomly selected fields. A: Control C33A; B: C33A transfected with NC mimics; C: C33A transfected with miR-214-3p mimics; D: C33A overexpressing control plasmid; E: C33A overexpressing THBS2; F: C33A overexpressing miR-214-3p mimics and THBS2. *, P≤0.05; ***, P≤0.01.

Discussion

Dysregulation of miRNAs has been demonstrated in a diverse number of human cancers. Accumulating evidence have revealed the regulatory role of miRNAs in tumour metastasis, cancer cell migration and invasion (23-25).

Previously, numerous studies reported that miR-214-3p plays strong tumorigenic and tumour suppressive activities in many human cancers (25, 26). miR-214-3p has a low expression level in various human cancers. However, the function that miR-214 plays in cervical cancer is still not clear with controversial results from previous studies in Hela cells (27, 28). In our study, decreasing expression of miR-214-3p was clearly identified in human cervical cancer cell line C33A and suppressive function of miR-214-3p was also confirmed in our study. The transfection of miR-214-3p mimics into C33A cells led to a significant decrease in cell viability, invasion as well as cell migration. Together with the low expression of miR-214-3p in normal cervical cells we detected, our results revealed that miR-214-3p exhibits strong tumour suppressive functions in cervical cancers.

So far, a serious of genes were identified as targets of miR-214-3p, including ARL2, FOXM1, HMGA1 and EZH2 (28-31). To further understand miR-214-3p in cervical cancer and reveal the mechanism underlying, bioinformatics analysis detected THBS2 as a novel potential target of miR-214 in our study. In a previous study, THBS2 mediates cell-to-cell and cell-to-matrix interactions and promotes cancer metastasis (32-34). In addition, the downregulation of THBS2 reduced MMP2/MMP9 degradation and promoted VEGF production (21, 22, 35-37). In the current study, overexpression of miR-214-3p inhibited THBS2, subsequently down-regulated protein levels of MMP2 and MMP9, resulting in a decreased cell migration and invasion ability in the C33A cell line.

The current study sheds light on the role of miR-214-3p in cervical cancer and its potential implications for diagnosis and treatment. We observed that miR-214-3p expression was significantly reduced in the cervical cancer cell line (C33A), indicating its involvement in the development and progression of the disease. Through experimental overexpression of miR-214-3p, we demonstrated its inhibitory effects on cell viability, invasion, and migration. These findings suggest that miR-214-3p acts as a tumor suppressor in cervical cancer by limiting the aggressive behavior of cancer cells. Furthermore, we identified THBS2 as a novel target gene of miR-214-3p. THBS2, which was found to be highly expressed in cervical cancer cells, plays a crucial role in cancer progression and metastasis. By targeting the 3' untranslated region (3'UTR) of THBS2, miR-214-3p effectively downregulated the protein level of THBS2. This regulatory mechanism provides a molecular basis for the observed inhibitory effects of miR-214-3p on cervical cancer cell behavior. The identification of THBS2 as a downstream target of miR-214-3p expands our understanding of the molecular pathways involved in cervical cancer.

Conclusion

Overall, our findings suggest that manipulating miR-

214-3p levels or targeting THBS2 could be potential strategies for the diagnosis and treatment of cervical cancer. Further investigations and clinical studies are warranted to validate the clinical relevance of miR-214-3p and its downstream target THBS2 in cervical cancer patients. In summary, our study contributes to the growing body of knowledge on miRNA-mediated regulation in cervical cancer and provides valuable insights into the potential therapeutic avenues that can be explored in the future.

Ethical Compliance
Not applicable.

Conflict of Interests
The authors declared no conflict of interest.

Acknowledgements
This study was supported by the 2021 Medical Science Research Project of Hebei Province (No: 20211215)

Author contribution
Xiaohua Wu and Xu Wang designed and directed the project; Xu Wang, Jing Xu, Fang Hua, Yujing Wang, Guiying Fang and Hongzhen Zhang performed the experiments. Xiaohua Wu and Xu Wang analyzed the data and wrote the manuscript.

References

1. Noordhuis MG, Eijsink JJ, Roossink F, et al. Prognostic cell biological markers in cervical cancer patients primarily treated with (chemo)radiation: a systematic review. Int J Radiat Oncol 2011; 79(2): 325-334.
2. Yang L, Luo X, Li X, Sun Y, Feng Y, Liu J. Arenobufagin Enhances the Radiosensitivity of Cervical Cancer Cells by Inhibiting the NF-?B Signaling Pathway. J Biomed Nanotechnol 2022; 18(5): 1488-1496.
3. Peiretti M, Zapardiel I, Zanagnolo V, Landoni F, Morrow CP, Maggioni A. Management of recurrent cervical cancer: a review of the literature. Surg Oncol 2012; 21(2): e59-e66.
4. Cetin I, Topcul M. Antiproliferative Effects of EGFR inhibitor Cetuximab and PARP Inhibitor Combination on Non-Small Cell Lung Cancer Cell Line A549 and Cervical Cancer Cell Line HeLa. Cell Mol Biol 2022; 68(8): 47-51.
5. He S, Yu G, Peng K, Liu S. MicroRNA-145-5p suppresses fascin to inhibit the invasion and migration of cervical carcinoma cells. Mol Med Rep 2020; 22(6): 5282-5292.
6. Zhang XY, Dong XM, Wang FP. MiR-377-3p inhibits cell metastasis and epithelial-mesenchymal transition in cervical carcinoma through targeting SGK3. Eur Rev Med Pharmaco 2020; 24(9): 4687-4696.
7. Pan X, Cao YM, Liu JH, Ding J, Xie XY, Cao PG. MEG3 Induces Cervical Carcinoma Cells' Apoptosis Through Endoplasmic Reticulum Stress by miR-7-5p/STC1 Axis. Cancer Biother Radio 2021; 36(6): 501-510.
8. Yang H, Kong W, He L, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res 2008; 68(2): 425-433.
9. Xiong X, Ren HZ, Li MH, Mei JH, Wen JF, Zheng CL. Down-regulated miRNA-214 induces a cell cycle G1 arrest in gastric cancer cells by up-regulating the PTEN protein. Pathol Oncol Res 2011; 17(4): 931-937.
10. Derfoul A, Juan AH, Difilippantonio MJ, Palanisamy N, Ried T, Sartorelli V. Decreased microRNA-214 levels in breast cancer cells coincides with increased cell proliferation, invasion and accumulation of the Polycomb Ezh2 methyltransferase. Carcinogenesis 2011; 32(11): 1607-1614.
11. Penna E, Orso F, Cimino D, et al. microRNA-214 contributes to melanoma tumour progression through suppression of TFAP2C. Embo J 2011; 30(10): 1990-2007.
12. Iruela-Arispe ML, Luque A, Lee N. Thrombospondin modules and angiogenesis. Int J Biochem Cell B 2004; 36(6): 1070-1078.
13. Ao R, Guan L, Wang Y, Wang JN. Silencing of COL1A2, COL6A3, and THBS2 inhibits gastric cancer cell proliferation, migration, and invasion while promoting apoptosis through the PI3k-Akt signaling pathway. J Cell Biochem 2018; 119(6): 4420-4434.
14. Tian Q, Liu Y, Zhang Y, et al. THBS2 is a biomarker for AJCC stages and a strong prognostic indicator in colorectal cancer. J Buon 2018; 23(5): 1331-1336.
15. Sun R, Wu J, Chen Y, et al. Down regulation of Thrombospondin2 predicts poor prognosis in patients with gastric cancer. Mol Cancer 2014; 13(225.
16. Qu HL, Hasen GW, Hou YY, Zhang CX. THBS2 promotes cell migration and invasion in colorectal cancer via modulating Wnt/beta-catenin signaling pathway. Kaohsiung J Med Sci 2022; 38(5): 469-478.
17. Huang TL, Mei YW, Li Y, et al. Thrombospondin-2 promotes the proliferation and migration of glioma cells and contributes to the progression of glioma. Chin Neurosurg J 2022; 8(1): 39.
18. Liao X, Wang W, Yu B, Tan S. Thrombospondin-2 acts as a bridge between tumor extracellular matrix and immune infiltration in pancreatic and stomach adenocarcinomas: an integrative pan-cancer analysis. Cancer Cell Int 2022; 22(1): 213.
19. Chen PC, Tang CH, Lin LW, et al. Thrombospondin-2 promotes prostate cancer bone metastasis by the up-regulation of matrix metalloproteinase-2 through down-regulating miR-376c expression. J Hematol Oncol 2017; 10(1): 33.
20. Liu JF, Chen PC, Chang TM, Hou CH. Thrombospondin-2 stimulates MMP-9 production and promotes osteosarcoma metastasis via the PLC, PKC, c-Src and NF-kappaB activation. J Cell Mol Med 2020; 24(21): 12826-12839.
21. Yang Z, Strickland DK, Bornstein P. Extracellular matrix metalloproteinase 2 levels are regulated by the low density lipoprotein-related scavenger receptor and thrombospondin 2. J Biol Chem 2001; 276(11): 8403-8408.
22. Jiao H, Zeng L, Zhang J, Yang S, Lou W. THBS2, a microRNA-744-5p target, modulates MMP9 expression through CUX1 in pancreatic neuroendocrine tumors. Oncol Lett 2020; 19(3): 1683-1692.
23. Zhu Z, Gong J, Kong J, et al. Iroquois Homeobox 5 Negatively Regulated by miRNA-147 Promotes the Proliferation, Metastasis, and Invasion by Oral Squamous Cell Carcinoma. J Biomed Nanotechnol 2021; 17(6): 1098-1108.
24. Zhu Y, Wu G, Yan W, Zhan H, Sun P. miR-146b-5p regulates cell growth, invasion, and metabolism by targeting PDHB in colorectal cancer. Am J Cancer Res 2017; 7(5): 1136-1150.
25. Li H, Qu L. Correlation of miRNA-21 and Blood Cr Levels with Tumor Infiltration and Distant Metastasis in Renal Cancer Patients. Cell Mol Biol 2022; 68(5): 117-123.
26. Zhang XJ, Ye H, Zeng CW, He B, Zhang H, Chen YQ. Dysregulation of miR-15a and miR-214 in human pancreatic cancer. J Hematol Oncol 2010; 3(46.
27. Yang Z, Chen S, Luan X, et al. MicroRNA-214 is aberrantly expressed in cervical cancers and inhibits the growth of HeLa cells. Iubmb Life 2009; 61(11): 1075-1082.
28. Yang Y, Liu Y, Li G, Li L, Geng P, Song H. microRNA-214 sup-

presses the growth of cervical cancer cells by targeting EZH2. Oncol Lett 2018; 16(5): 5679-5686.
29. Chandrasekaran KS, Sathyanarayanan A, Karunagaran D. MicroRNA-214 suppresses growth, migration and invasion through a novel target, high mobility group AT-hook 1, in human cervical and colorectal cancer cells. Brit J Cancer 2016; 115(6): 741-751.
30. Peng R, Men J, Ma R, et al. miR-214 down-regulates ARL2 and suppresses growth and invasion of cervical cancer cells. Biochem Bioph Res Co 2017; 484(3): 623-630.
31. Wang JM, Ju BH, Pan CJ, et al. MiR-214 inhibits cell migration, invasion and promotes the drug sensitivity in human cervical cancer by targeting FOXM1. Am J Transl Res 2017; 9(8): 3541-3557.
32. Wang X, Zhang L, Li H, Sun W, Zhang H, Lai M. THBS2 is a Potential Prognostic Biomarker in Colorectal Cancer. Sci Rep-Uk 2016; 6: 33366.
33. Liu QH, Ma LS. Knockdown of thrombospondin 2 inhibits metastasis through modulation of PI3K signaling pathway in uveal melanoma cell line M23. Eur Rev Med Pharmaco 2018; 22(19): 6230-6238.
34. Ng KY, Shea QT, Wong TL, et al. Chemotherapy-Enriched THBS2-Deficient Cancer Stem Cells Drive Hepatocarcinogenesis through Matrix Softness Induced Histone H3 Modifications. Adv Sci 2021; 8(5): 2002483.
35. Azizi Dargahlou, S., Iriti, M., Pouresmaeil, M., Goh, L. P. W. MicroRNAs; their therapeutic and biomarker properties. Cell Mol Biomed Rep 2023; 3(2): 73-88. doi: 10.55705/cmbr.2022.365396.1085
36. Kanwal, N., Al Samarrai, O., Al-Zaidi, H. M. H., Mirzaei, A., Heidari, M. Comprehensive analysis of microRNA (miRNA) in cancer cells. Cell Mol Biomed Rep 2023; 3(2): 89-97. doi: 10.55705/cmbr.2022.364591.1070.
37. Lawler J, Detmar M. Tumor progression: the effects of thrombospondin-1 and -2. Int J Biochem Cell B 2004; 36(6): 1038-1045.