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MiR-32-5p regulates the proliferation and metastasis of cervical cancer cells by targeting HOXB8

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Abstract. – OBJECTIVE: To investigate the effects of miR-32-5p on the biological behaviors of cervical cancer (CCa), the relevant mechanism was studied in CCa cell lines (HeLa) *in vitro*.

PATIENTS AND METHODS: The expression level of miR-32-5p was detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR). TargetScan, miRDB, microRNA databases and Luciferase method were conducted to predict and validate the target gene of miR-32-5p; the effects of miR-32-5p on cell proliferation, clone formation, invasion and migration capacity were analyzed *in vitro* study.

RESULTS: We found miR-32-5p to be significantly inhibited in CCa tissues and cells. Bioinformatics approach together with Luciferase method screened Homeobox B8 (HOXB8) as a downstream regulatory target of miR-32-5p. Besides, HOXB8 was incredibly high expression in CCa tissues and cells. After transfection in HeLa cells by miR-32-5p mimics, HOXB8 expression was indicated to be negatively correlated with miR-32-5p both in qRT-PCR and Western blot (WB) assays. The subsequent experiments showed that decreased expression of HOXB8 resulting from up-regulation of miR-32-5p could weaken the cell proliferation, clone formation, invasion and migration ability of HeLa cells.

CONCLUSIONS: MiR-32-5p could inhibit the cellular malignant behavior through regulating the expression of HOXB8 in HeLa cells. We provide a new clue for the study of molecular mechanisms of CCa. MiR-32-5p/HOXB8 axis might serve as potential target for the clinical diagnosis and treatment of CCa.

Key Words:
MicroRNA-32-5p (miR-32-5p), Cervical cancer (CCa), Homeobox B8 (HOXB8).

Introduction

Cervical cancer (CCa) is one of the most common female cancers in the world, especially in China[1-3]. According to incomplete statistics of the World Health Organization, there were approximately 530,000 new CCa patients every year around the world, and the number increased progressively year by year at a rate of 5% of total female population, showing a younger trend[4, 5]. At the same time, about 270,000 people died of CCa every year, and its cancer mortality ranked third[6,7]. Although the major risk factor for human-induced CCa in clinic was the persistent infection of high-risk human papillomavirus (HPV)[8,9], evidence suggested that the simple infection of HPV was insufficient to induce the malignant transformation of normal cervix alone, which might be caused by changes in other genes in CCa[4,10]. Therefore, the molecular mechanism of CCa invasiveness remains to be further clarified and confirmed using more tumor-specific molecular markers. Micro ribonucleic acid (miRNA) is a kind of universally-expressed endogenous short-chain non-coding RNA molecular fragment with targeted intervention in the post-transcriptional regulation of 3'-untranslated region (UTR) of the target gene, which exerts effects in the gene expression, post-transcriptional regulation, protein activity and expression regulation[11]. Researches had demonstrated that miRNA played an important role in a variety of diseases, including cancer, and was mainly involved in inhibiting the gene expression at the post-transcriptional level, thereby regulating the cell growth, proliferation, cell

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cycle regulation, differentiation and apoptosis[12-16]. Moreover, it was found in the study on CCa and its precancerous lesions that a large number of miRNAs were abnormally expressed and participated in the incidence and development of CCa through affecting the expression level of target genes[17-22]. MiR-32-5p, as one of the miRNA molecules, was associated with tumorigenesis in different cancer types including hepatocellular carcinoma (HCC)[23], clear cell renal cell carcinoma (ccRCC)[24] and pancreatic cancer (PC)[25]. However, few of evidence about the role of miR-32-5p in the development of CCa and its related molecular mechanisms were mentioned. Here, we analyzed the expression of miR-32-5p in CCa and investigated the effects of miR-32-5p on the biological behaviors of CCa cell lines. Furthermore, some related molecular mechanisms involved were described as well.

Patients and Methods

CCa Tissues and Cells

80 cases of human CCa tissues and adjacent normal tissues (more than 5 cm from tumor tissues were necessary) were collected from July 2015 year to April 2017 year in Affiliated Hospital of North Sichuan Medical College. Preoperative chemotherapy or radiotherapy treatment was forbidden. All patients had signed informed consent and Ethics Committee approval was given by our hospital. The CCa cell lines (siHa) together with normal human embryonic kidney cell lines (293T) were purchased from Shanghai University of Chinese Academy of Sciences Library (Shanghai, China). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (containing 10% fetal bovine serum (FBS), 100 μg/mL streptomycin and 100 IU/mL penicillin) (Gibco, Rockville, MD, USA) and placed in a 37°C, 5% CO_2 incubator. Cells in logarithmic growth phase were used for experiments.

Target Prediction and Luciferase Reporter Assays

Target biogene prediction of miR-32-5p was performed using bioinformatics prediction software TargetScan (http://www.targetscan.org), miRDB (http://www.mirdb.org/) and microRNA (http://www.microrna.org/), and appropriate target gene [Homeobox B8 (HOXB8)] was screened.

HeLa cells in logarithmic growth phase were seeded in 24-well plates. When the cell confluence reached 50-70%, transient transfection was performed according to the transfection reagent, miR-32-5p mimics/NC and pmirGLO-BMF plasmid (wt)/(mut) were synthesized and co-transfected into HeLa cells, respectively. Luciferase activity was detected in a multi-function microplate reader according to the dual luciferase activity assay kit instructions.

Transfection

MiR-NC, miR-32-5p mimics and plasmid pcDNA3.1-HOXB8 were synthesized by Genepharma and were transfected by using Lipofectamine RNAiMAX (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer's instructions. In brief, HeLa cells were seeded into 6-well plates and cultured to a cell density of 50-70%. 10 μL of miRNA-NC transfected cells were added to each well of the NC group, 10 μL of miR-32-5p mimics were added to each well of the mimics group, mimics + LV-HOXB8 group was transfected with plasmid pcDNA3.1-HOXB8 and miR-32-5p mimics.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR, qPCR) and Western blot (WB) Analysis

The expression levels of mRNA and protein were measured 48 h after transfection. For qRT-PCR analysis: total RNA was extracted from cell lines and tissue samples by TRIzol (Invitrogen, Carlsbad, CA, USA) and its concentration was quantitatively determined in accordance with the manufacturer's protocol. qRT-PCR analysis was performed using the SYBR green kit (TaKaRa, Otsu, Shiga, Japan). U6 snRNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) serve as internal references for miR-32-5p and HOXB8, respectively. Results obtained were calculated by formula ($RQ=2^{-\Delta\Delta Ct}$). For WB analysis: 48 h after transfection, the total protein was extracted from cells, and the protein concentration was detected using the bicinchoninic acid (BCA) protein quantitative detection kit (Pierce, Rockford, IL, USA). 20 μg proteins were taken for 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), sealed with 5% skim milk powder at room temperature for 3-4 h and washed with Tris-buffered saline and Tween 20 (TBST) for 10 min. Next, the mouse anti-human HOXB8 monoclonal primary antibody (1:1000) was added for incubation on the shaking table at 4°C over-

night. The horseradish peroxidase (HRP)-labeled goat anti-mouse secondary antibody (1:5000) was also added for incubation on the shaking table at 4°C for 2 h. The color was developed using the enhancedchemiluminescence (ECL) developing solution (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the value was standardized using the internal reference GAPDH band.

Cell Proliferation

HeLa cells were prepared into 1×10^4/mL cell suspension and inoculated into a 96-well plate (100 μL/well). Cells were divided into 3 groups, and 6 repeated wells were set in each group. At 24 h after transfection, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reagent (5 mg/mL) was added (10 μL/well) for incubation for another 3-4 h. After the culture solution was discarded, 150 μL dimethyl sulfoxide (DMSO) were added each well and vibrated on a shaking table at room temperature for 15 min. Finally, the absorbance value of each well was detected using an enzyme-linked immunosorbent assay instrument.

Clone Formation

24 h after transfection, cells in each group were digested, counted, prepared into 200/mL cell suspension and inoculated into a 6-well plate. 3 repeated wells were set in each group. After routine culture for 2 weeks, the culture was terminated when there were cell families visible to the naked eye. After that, cells were fixed in phosphate-buffered saline (PBS) containing 4% paraformaldehyde and stained with 0.1% crystal violet for 30 min. The excess dye was washed away with phosphate-buffered saline (PBS), and cells were counted under a microscope.

Cell Invasion and Migration Assays

Cell migration and invasion abilities were measured using a Transwell chamber (Corning, Corning, NY, USA) with a pore size of 8 μm, in which 500 μL of serum-free medium were added to the upper chamber while 800 μL of 1640 medium containing 10% fetal bovine serum (FBS) was added to the lower chamber. After 48 h of transfection, 5×10^5 cells were added to the chamber after they were counted; 24 h later incubation, the chamber was removed, and the unmigrated cells in the upper part of the chamber membrane were removed and fixed in ethanol for 15 min, followed by trypan blue staining. The cell invasion assay used a transwell chamber previously coated with matrigel glue and the remaining steps were basically the same as those in migration assay. Finally, five fields of view were randomly selected under an inverted microscope (×200) for counting, and the average was calculated.

Statistical Analysis

Statistical analysis was performed with a Student's *t*-test or *F*-test. All *p*-values were two-sided and $p<0.05$ were considered significant and analyzed by Prism 6.02 software (La Jolla, CA, USA).

Results

MiR-32-5p Expression Found Reduced Both in Tissues and Cells of CCa

QRT-PCR was used to detect the expression of miR-32-5p in CCa tissues and paired adjacent non-tumor tissues in 80 patients. The results showed that the expression of miR-32-5p was significantly oppressed in CCa tissues compared to the matched adjacent normal tissues (Figure 1A). Meanwhile, the expression level of miR-32-5p in CCa cell lines was consistent with the results of tissue inspection (Figure 1B). These results suggested that the expression of miR-32-5p was significantly down-regulated in CCa.

HOXB8 is a Direct Target of miR-32-5p in CC Cell

TargetScan (http://www.targetscan.org), miRDB (http://www.mirdb.org/) and microRNA (http://www.microrna.org/) bioinformatics approach databases were used to elucidate the putative and possible targets of miR-32-5p and HOXB8 was checked as a supposed target of miR-32-5p (Figure 2A). Immediately after the prediction, luciferase reporter assays, as the gold standard experiment for the identification of miRNA target genes, was performed. The results first clarified the achievable transfection efficiency of mimics (Figure 2B). The next core result showed that increased the expression of miR-32-5p with mimics resulted in the decrease of the luciferase activity of the wide-type, but it had no effect on mutant-type (Figure 2C). These findings demonstrated the direct targeting of miR-32-5p to HOXB8, confirming our hypothesis. Another gratifying result was that HOXB8 exhibited an abnormally high expression level in both CCa tissues and cell lines (Figure 2D-2E). Combined with the above results, it was hard not to further discuss the role of miR-32-5p and HOXB8 in CCa development. Therefore, three

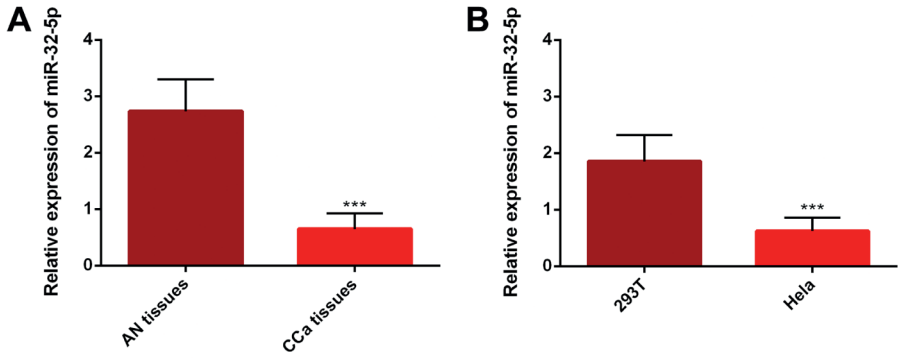

Figure 1. The expressions of miR-32-5p in cervical cancer (CCa) tissue samples and cells comparing with corresponding adjacent normal tissues and human normal embryonic kidney cells. ***A,*** Difference in the expression of miR-32-5p in tissues. (***p<0.0001 compared with adjacent normal tissue). ***B,*** Difference in the expression of miR-32-5p in cells. (***p<0.001 compared with 293T).

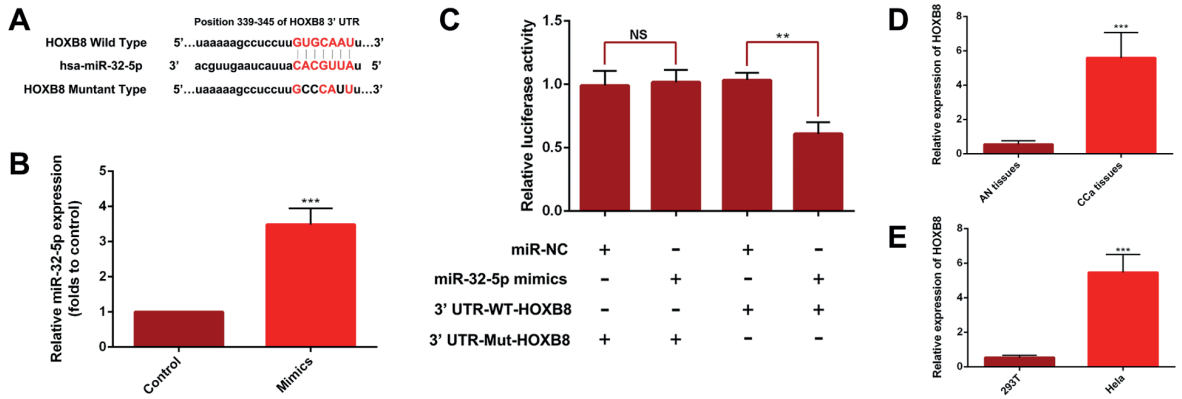

Figure 2. HOXB8 is a direct and functional target of miR-32-5p. ***A,*** Diagram of putative miR-32-5p binding sites of HOXB8. ***B,*** Transfection efficiency detection by qRT-PCR. ***C,*** Relative activities of luciferase reporters. ***D-E,*** The expressions of HOXB8 in CCa tissues and cells, Data were presented as means ± standard deviations. (**p<0.01, ***p<0.001).

experimental groups were established and used for further *in vitro* experiments

MiR-32-5p Decreased the Expression Level of HOXB8

The results in qRT-PCR and WB analysis showed that the expression of HOXB8 mRNA and protein were significantly down-regulated after overexpression of miR-32-5p in HeLa cells (Figure 3A-3B). This result further illustrated the negative regulation of miR-32-5p on HOXB8 expression.

MiR-32-5p Suppressed Proliferation of CC Cell

After transfection into HeLa cells, the changes in the proliferation ability of HeLa cells were detected *via* MTT assay and colony formation assay. Results revealed that the proliferation and colony formation abilities of HeLa cells significantly declined after overexpression of miR-32-5p compared with those in negative control group, while the proliferation ability of HeLa cells was significantly increased after the HOXB8 expression was restored compared with that in miR-32-5p mimics group (Figure 3C-3E).

MiR-32-5p Inhibited Invasion and Migration of CCa cell

Migration and invasion are two most key factors in cancer cell metastasis[26]. We examined the migration and invasion abilities of cells after up-regulation of miR-32-5p in HeLa cells. The results from Transwell experiment showed that the migration and invasion abilities of miR-32-5p overexpressed HeLa cells were significantly

weakened, and the number of migratory and invasive cells was significantly lower than that of the NC group. However, after restoring the expression of HOXB8, the effects of miR-32-5p were severely alleviated (Figure 4).

Discussion

CCa is one of the most common malignant tumors in the female reproductive system[27]. At present, the high rate of distant lymphatic metastasis of CCa restricted the clinical diagnosis and treatment[28, 29]. The number of CCa patients gradually increased due to the lack of effective biomarkers, which had become a concerned issue for research scholars[30-32]. The survival rate and vital signs of CCa patients could be significantly prolonged and improved *via* surgery and chemoradiotherapy, but unfortunately its clinical treatment was very limited due to the high rate of distant metastasis and low survival rate of CCa patients. As many as 17% women would often suffered from recurrence *in situ* and metastasis in the first 2 years after treatment, and the postoperative 5-year survival rate of elderly patients with advanced CCa or distant metastasis was less than 20%[33,34]. Moreover, these treatment methods, due to poor specificity, different patient's constitution and difficulty in dose control, often caused great damage to patients and lead to serious side reactions, which lack the potential value of long-term application[35-37]. To further change the increasing incidence rate of CC and improve the targeted therapeutic regimen for CCa, therefore, identifying the CCa-related tumor genes with tissue specificity, might become a new theoretical and technical means of diagnosis and treatment. MiRNA is a kind of highly-conserved single-stranded non-coding small RNA[11], and it had become one of the hotspots in tumor research in recent years. Researches had demonstrated that miRNA, accounting for only 1% of the human genome, regulates the expression of about 60% human protein-coding genes[38,39]. Approximately 50% miRNA genes were located in the fragile site of tumor[40], which were closely related to the occurrence and development of tumor. The interaction between miRNA and its target genes formed a complex regulatory network, so exploring and verifying related target genes of miRNA was the core in the miRNA research. In our study, the downstream genes possibly regulated by miR-32-5p were predicted firstly, and the bioinformatics software showed that there were complementary base sequences between miR-32-5p and 3'-UTR of HOXB8, theoretically proving that miR-32-5p

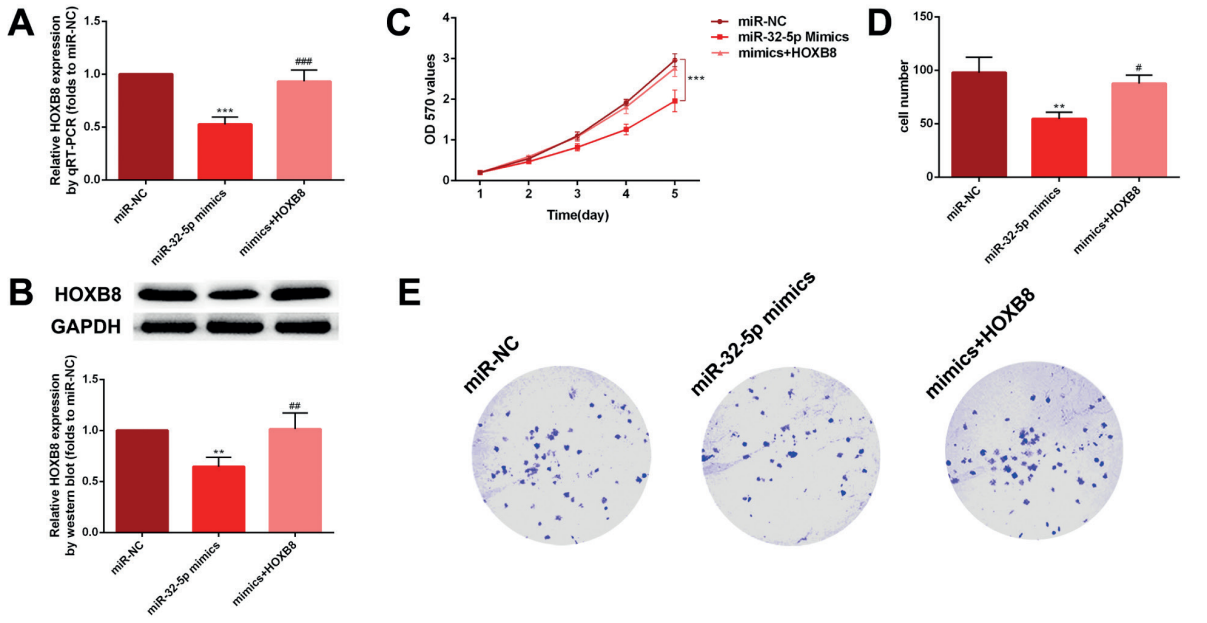

Figure 3. ***A,*** MiR-32-5p decreased the mRNA expression level of HOXB8, ***B,*** MiR-32-5p decreased the protein expression level of HOXB8. ***C,*** MiR-32-5p inhibited the proliferation of CCa cells. ***D-E,*** Assessment of colony formation, Data were presented as means ± standard deviations. ($^{**}p<0.01$, $^{***}p<0.001$ *vs.* NC group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ *vs.* Mimics group).

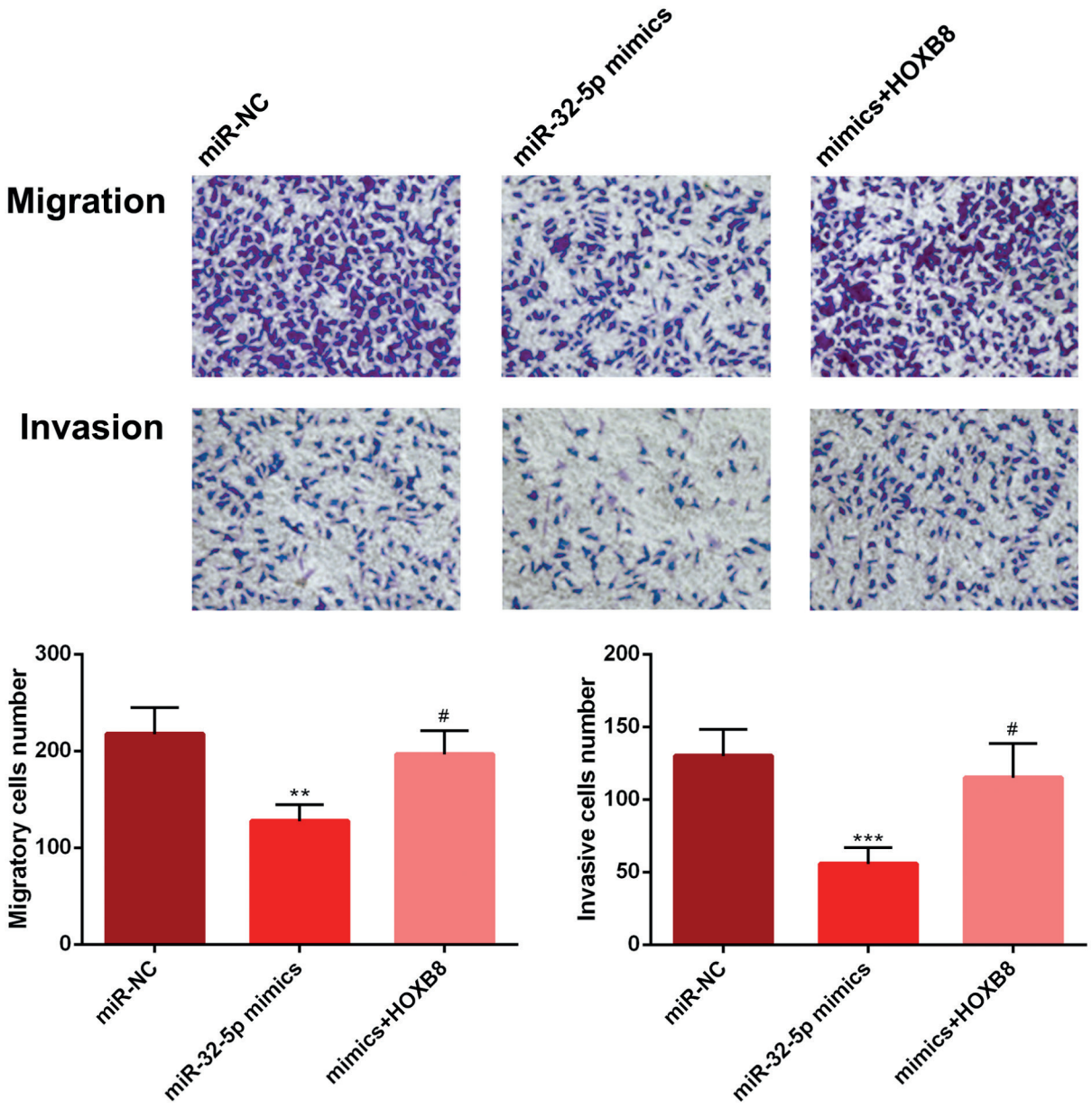

Figure 4. The invasion and migration of CCa cells were analyzed using transwell assay and detected by microscope (Magnification × 200). Data were presented as means ± standard deviations. (**p<0.01, ***p<0.001 *vs.* NC group; #p<0.05 *vs.* Mimics group).

might regulate the expression of HOXB8 gene. In order to detect whether miR-32-5p could directly regulated the HOXB8 gene, dual-luciferase reporter gene system was used for verification, and it was found that increased the expression of miR-32-5p leading to the decrease of the luciferase activity of the wide-type, but it had no effect on mutant-type, indicating that there were specific complementary pairing sequences, as well as a direct targeted relation between base sequence of miR-32-5p and HOXB8 mRNA 3'-UTR. The HOXB8 gene is a member of the Hox gene family, which is located on chromosome 17 and encodes the nucleoprotein of homeobox DNA-binding domain[41]. Moreover, it was also a sequence-specific transcription factor that played an important role in cell differentiation, development and organ formation[42-44]. In recent years, the study on HOXB8 mainly focused on its development and pathogenic mechanism in the nervous system, respiratory system and blood system[45-48]. In 2008, Rawat et al[49] found that in the mouse model, the

deletion of N-terminal domain of caudal-type homeobox 2 (CDX2) could lead to the loss of Hox gene expression, thus resulting in the abnormal differentiation of hematopoietic stem cells and causing acute myeloid leukemia (AML) in mice. Therefore, Shen et al[50] had gradually explored the HOXB8 gene in the tumor-related study in recent years and made some achievements. For example, HOXB8 was found associated with chemotherapy resistance in human colorectal cancer by SHEN. In gastric cancer research, DING found HOXB8 exerted promoting effects on tumor development[51]. Similarly, HOXB8 herald a short survival in ovarian serous carcinoma, a result obtained by Stavnes et al[52]. However, the role of HOXB8 in CCa and its mechanism were rarely reported. The preliminary clinical research revealed that miR-32-5p was significantly inhibited in CCa tissues, while the expression of HOXB8 gene was significantly increased in CCa tissues. It was also observed in target gene detection that miR-32-5p could regulated the expression of HOXB8 in CCa in a targeted manner, so it was scientifically speculated that the miR-32-5p/HOXB8 axis exerted a regulatory effect in the development of CCa. In view of this, miR-NC (for control), HOXB8 and/or miR-32-5p mimics were transfected into HeLa cells to observe changes in the cell function. As expected, the relative expression levels of HOXB8 mRNA and protein significantly declined after overexpression of miR-32-5p in HeLa cells, further proving that miR-32-5P could regulate the expression of HOXB8 in HeLa cells in a targeted manner. Then, it was found in the functional experiment that the proliferation, clone formation, invasion and migration abilities of HeLa cells with low expression of HOXB8 were significantly lower than those in control group, and the malignant behaviors of HeLa cells were significantly inhibited. Notably, after HOXB8 and miR-32-5p mimics were co-transfected into HeLa cells, the malignant behaviors of HeLa cells were seemingly awakened, and its proliferation, clone formation, invasion and migration abilities were almost the same as those in control group.

Conclusions

We showed that the regulation of the expression of HOXB8 by miR-32-5p suppressed the malignant behavior of the CCa cells. As such, miR-32-5p acts as a tumor suppressor gene in CCa. This result would provide experimental evidence for finding new therapeutic targets for CCa treatment.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Sun R, Zhang H, Liu K, Fan J, Li G, Song X, Li C. Clinicopathologic predictive factors of cervical lymph node metastasis in differentiated thyroid cancer. Acta Otorrinolaringol Esp 2018; 69: 149-155.
2) Ismail E, Kornovski Y, Dimitrov T. [A case of isolated massive pre-sacral lymph node metastasis in 1B1 stage cervical cancer - case report.]. Akush Ginekol (Sofiia) 2016; 55: 44-49.
3) Becerra-Culqui TA, Lonky NM, Chen Q, Chao CR. Patterns and correlates of cervical cancer screening initiation in a large integrated health care system. Am J Obstet Gynecol 2018; 218: 421-429.
4) Mazdziarz A, Wygledowski J, Osuch B, Jagielska B, Spiewankiewicz B. New directions in cervical cancer prophylaxis worldwide and in Poland - case study of the Polish rural female population. Ann Agric Environ Med 2017; 24: 592-595.
5) Ronco G, Franceschi S. Cervical cancer screening: the transformational role of routine human papillomavirus testing. Ann Intern Med 2018; 168: 75-76.
6) Ma C, Zhang Y, Li R, Mao H, Liu P. Risk of parametrial invasion in women with early stage cervical cancer: a meta-analysis. Arch Gynecol Obstet 2018; 297: 573-580.
7) Aviles-Jimenez F, Yu G, Torres-Poveda K, Madrid-Marina V, Torres J. On the search to elucidate the role of microbiota in the genesis of cancer: the cases of gastrointestinal and cervical cancer. Arch Med Res 2017; 48: 754-765.
8) Liu G, Sharma M, Tan N, Barnabas RV. HIV-positive women have higher risk of human papilloma virus infection, precancerous lesions, and cervical cancer. AIDS 2018; 32: 795-808.
9) Alifu M, Fan P, Kuerban G, Yao X, Peng Y, Dong T, Wang R. Frequency distribution of HLA alleles and haplotypes in Uyghur women with advanced squamous cell cervical cancer and relation to HPV status and clinical outcome. Arch Gynecol Obstet 2018; 297: 757-766.
10) AL-Hammadi FA, Al-Tahri F, Al-Ali A, Nair SC, Abdulrahman M. Limited understanding of pap smear testing among women, a barrier to cervical cancer screening in the United Arab Emirates. Asian Pac J Cancer Prev 2017; 18: 3379-3387.
11) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
12) Wu W, Sun M, Zou GM, Chen J. MicroRNA and cancer: current status and prospective. Int J Cancer 2007; 120: 953-960.

13) Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. Development 2005; 132: 4653-4662.

14) Croce CM, Calin GA. miRNAs, cancer, and stem cell division. Cell 2005; 122: 6-7.

15) Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.

16) Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005; 65: 7065-7070.

17) Lee JW, Choi CH, Choi JJ, Park YA, Kim SJ, Hwang SY, Kim WY, Kim TJ, Lee JH, Kim BG, Bae DS. Altered MicroRNA expression in cervical carcinomas. Clin Cancer Res 2008; 14: 2535-2542.

18) Hu X, Schwarz JK, Lewis JJ, Huettner PC, Rader JS, Deasy JO, Grigsby PW, Wang X. A microRNA expression signature for cervical cancer prognosis. Cancer Res 2010; 70: 1441-1448.

19) Wilting SM, van Boerdonk RA, Henken FE, Meijer CJ, Diosdado B, Meijer GA, le Sage C, Agami R, Snijders PJ, Steenbergen RD. Methylation-mediated silencing and tumour suppressive function of hsa-miR-124 in cervical cancer. Mol Cancer 2010; 9: 167.

20) Wang F, Li Y, Zhou J, Xu J, Peng C, Ye F, Shen Y, Lu W, Wan X, Xie X. miR-375 is down-regulated in squamous cervical cancer and inhibits cell migration and invasion via targeting transcription factor SP1. Am J Pathol 2011; 179: 2580-2588.

21) Xu J, Li Y, Wang F, Wang X, Cheng B, Ye F, Xie X, Zhou C, Lu W. Suppressed miR-424 expression via upregulation of target gene Chk1 contributes to the progression of cervical cancer. Oncogene 2013; 32: 976-987.

22) Wei H, Zhang JJ, Tang QL. MiR-638 inhibits cervical cancer metastasis through Wnt/beta-catenin signaling pathway and correlates with prognosis of cervical cancer patients. Eur Rev Med Pharmacol Sci 2017; 21: 5587-5593.

23) Fu X, Liu M, Qu S, Ma J, Zhang Y, Shi T, Wen H, Yang Y, Wang S, Wang J, Nan K, Yao Y, Tian T. Exosomal microRNA-32-5p induces multidrug resistance in hepatocellular carcinoma via the PI3K/Akt pathway. J Exp Clin Cancer Res 2018; 37: 52.

24) Wang M, Sun Y, Xu J, Lu J, Wang K, Yang DR, Yang G, Li G, Chang C. Preclinical studies using miR-32-5p to suppress clear cell renal cell carcinoma metastasis via altering the miR-32-5p/TR4/HGF/Met signaling. Int J Cancer 2018; 143: 100-112.

25) Gao ZQ, Wang JF, Chen DH, Ma XS, Wu Y, Tang Z, Dang XW. Long non-coding RNA GAS5 suppresses pancreatic cancer metastasis through modulating miR-32-5p/PTEN axis. Cell Biosci 2017; 7: 66.

26) Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer 2003; 3: 362-374.

27) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

28) Huchko MJ, Kahn JG, Smith JS, Hiatt RA, Cohen CR, Bukusi E. Study protocol for a cluster-randomized trial to compare human papillomavirus based cervical cancer screening in community-health campaigns versus health facilities in Western Kenya. Bmc Cancer 2017; 17: 826.

29) Lai A, Perucho J, Xu X, Hui ES, Lee E. Concordance of FDG PET/CT metabolic tumour volume versus DW-MRI functional tumour volume with T2-weighted anatomical tumour volume in cervical cancer. BMC Cancer 2017; 17: 825.

30) Li X, Zheng R, Li X, Shan H, Wu Q, Wang Y, Chen W. Trends of incidence rate and age at diagnosis for cervical cancer in China, from 2000 to 2014. Chin J Cancer Res 2017; 29: 477-486.

31) Shu T, Zhao D, Li B, Wang Y, Liu S, Li P, Zuo J, Bai P, Zhang R, Wu L. Prognostic evaluation of postoperative adjuvant therapy for operable cervical cancer: 10 years' experience of National Cancer Center in China. Chin J Cancer Res 2017; 29: 510-520.

32) Rerucha CM, Caro RJ, Wheeler VL. Cervical Cancer Screening. Am Fam Physician 2018; 97: 441-448.

33) Amkreutz L, Pijnenborg J, Joosten D, Mertens H, Van Kuijk S, Engelen M, Bergmans M, Nolting WE, Kruitwagen R. Contribution of cervical cytology in the diagnostic work-up of patients with endometrial cancer. Cytopathology 2018; 29: 63-70.

34) Lucia F, Visvikis D, Desseroit MC, Miranda O, Malhaire JP, Robin P, Pradier O, Hatt M, Schick U. Prediction of outcome using pretreatment (18) F-FDG PET/CT and MRI radiomics in locally advanced cervical cancer treated with chemoradiotherapy. Eur J Nucl Med Mol Imaging 2018; 45: 768-786.

35) Ye GL, Du DL, Jin LJ, Wang LL. Sensitization of TRAIL-resistant cervical cancer cells through combination of TRAIL and fucoxanthin treatments. Eur Rev Med Pharmacol Sci 2017; 21: 5594-5601.

36) Yang S, Jia C, Zhu H, Han S. CYP1A1 Ile462Val polymorphism and cervical cancer: evidence from a meta-analysis. Tumour Biol 2012; 33: 2265-2272.

37) Yamada M, Uchiyama K, Tashiro M. [A case of cervical lymph node metastasis from an unknown primary cancer controlled with immunotherapy, chemotherapy, and surgery]. Gan To Kagaku Ryoho 2017; 44: 2113-2116.

38) Tsai LM, Yu D. MicroRNAs in common diseases and potential therapeutic applications. Clin Exp Pharmacol Physiol 2010; 37: 102-107.

39) Hata A, Lieberman J. Dysregulation of microRNA biogenesis and gene silencing in cancer. Sci Signal 2015; 8: e3.

40) Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001; 294: 853-858.

41) Yue Y, Farcas R, Thiel G, Bommer C, Grossmann B, Galetzka D, Kelbova C, Kupferling P, Daser A, Zechner U, Haaf T. De novo t(12;17)(p13.3;q21.3) translocation with a breakpoint near the 5' end of the HOXB gene cluster in a patient with developmental delay and skeletal malformations. Eur J Hum Genet 2007; 15: 570-577.

42) Greer JM, Capecchi MR. Hoxb8 is required for normal grooming behavior in mice. Neuron 2002; 33: 23-34.

43) Fujimura Y, Isono K, Vidal M, Endoh M, Kajita H, Mizutani-Koseki Y, Takihara Y, van Lohuizen M, Otte A, Jenuwein T, Deschamps J, Koseki H. Distinct roles of polycomb group gene products in transcriptionally repressed and active domains of Hoxb8. Development 2006; 133: 2371-2381.

44) Sakaguchi S, Nakatani Y, Takamatsu N, Hori H, Kawakami A, Inohaya K, Kudo A. Medaka unextended-fin mutants suggest a role for Hoxb8a in cell migration and osteoblast differentiation during appendage formation. Dev Biol 2006; 293: 426-438.

45) Fanarraga ML, Charite J, Hage WJ, De Graaff W, Deschamps J. Hoxb-8 gain-of-function transgenic mice exhibit alterations in the peripheral nervous system. J Neurosci Methods 1997; 71: 11-18.

46) Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, Capecchi MR. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. Cell 2010; 141: 775-785.

47) Grier DG, Thompson A, Lappin TR, Halliday HL. Quantification of Hox and surfactant protein-B transcription during murine lung development. Neonatology 2009; 96: 50-60.

48) Huber L, Ferdin M, Holzmann J, Stubbusch J, Rohrer H. HoxB8 in noradrenergic specification and differentiation of the autonomic nervous system. Dev Biol 2012; 363: 219-233.

49) Rawat VP, Thoene S, Naidu VM, Arseni N, Heilmeier B, Metzeler K, Petropoulos K, Deshpande A, Quintanilla-Martinez L, Bohlander SK, Spiekermann K, Hiddemann W, Feuring-Buske M, Buske C. Overexpression of CDX2 perturbs HOX gene expression in murine progenitors depending on its N-terminal domain and is closely correlated with deregulated HOX gene expression in human acute myeloid leukemia. Blood 2008; 111: 309-319.

50) Shen S, Pan J, Lu X, Chi P. Role of miR-196 and its target gene HoxB8 in the development and proliferation of human colorectal cancer and the impact of neoadjuvant chemotherapy with FOLFOX4 on their expression. Oncol Lett 2016; 12: 4041-4047.

51) Ding WJ, Zhou M, Chen MM, Qu CY. HOXB8 promotes tumor metastasis and the epithelial-mesenchymal transition via ZEB2 targets in gastric cancer. J Cancer Res Clin Oncol 2017; 143: 385-397.

52) Stavnes HT, Holth A, Don T, Kaern J, Vaksman O, Reich R, Trope' CG, Davidson B. HOXB8 expression in ovarian serous carcinoma effusions is associated with shorter survival. Gynecol Oncol 2013; 129: 358-363.