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MiR-129-5p is downregulated in breast cancer cells partly due to promoter H3K27m3 modification and regulates epithelial-mesenchymal transition and multi-drug resistance

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Abstract. – OBJECTIVE: In this study, we firstly studied whether H3K27me3 modification is a mechanism of miR-129-5p downregulation in breast cancer and further investigated the functional role of miR-129-5p in epithelial-to-mesenchymal transition (EMT) and in multi-drug resistance (MDR) of the cancer cells.

MATERIALS AND METHODS: Immunoprecipitation (IP) and Chromatin Immunoprecipitation (ChIP) assay were performed to detect the association among SOX4, EZH2 and H3K27me3 and their enrichment in the promoter region of miR-129-2. Western blot and immunofluorescent staining were performed to detect the expression of epithelial and mesenchymal markers. MTT assay was applied to test drug sensitivity.

RESULTS: Enforced EZH2 and SOX4 expression resulted in suppressed miR-129-5p level in MCF-7 cells. There was an interaction among SOX4, EZH2 and H3K27me3 modification and they were significantly enriched in the region upstream of transcription start of miR-129-2. MCF-7 cells transfected with miR-129-5p mimics had significantly suppressed SOX4 expression. MCF-7 cells with miR-129-5p overexpression had significantly restored E-cadherin expression and suppressed N-cadherin and Vimentin expression. The drug sensitivity assay showed that miR-129-5p substantially reduced IC50 of ADM, VCR and PTX in MCF-7/ADM cells

CONCLUSIONS: There is a reciprocal regulation between miR-129-5p and SOX4 via the SOX4/EZH2 complex mediated H3K27me3 modification in breast cancer cells. MiR-129-5p is an important miRNA modulating EMT and MDR in breast cancer cells.

Key Words:
MiR-129-5p, Breast cancer, H3K27m3, EMT, MDR.

Introduction

Epigenetic dysregulation is closely related to carcinogenesis[1-3]. There are emerging evidence showed that many oncogenes and tumor suppressive genes in breast cancer are dysregulated as a result of epigenetic modification[4]. MicroRNAs (miRNAs) is a group of small, conserve and non-coding RNA that regulates gene expression via inducing mRNA degradation or suppressing protein translation by binging to the 3' or 5' untranslated region (UTR) of the targeting gene[5]. A series of tumor suppressive miRNAs are downregulated in breast cancer due to epigenetic mechanisms, leading to alteration of biological properties of the cancer cells, such as epithelial to mesenchymal transition (EMT) and enhanced resistance to chemotherapeutic drugs[4,6,7]. For example, DNA methylation and histone deacetylation are responsible for miR-375 downregulation in breast cancer, which subsequently result in trastuzumab resistance in HER2-positive breast cancer by increasing the expression of miR-375 targeting gene IGF1R[8]. Hypermethylation regulated downregulation of miR-149 can enhance chemoresistance of breast cancer cells via increasing the expression of GlcNAc N-deacetylase/N-sulfotransferase-1[9].

MiR-129-5p is transcribed from two genes, miR-129-1 and miR-129-2, which were located on chromosome 7 and chromosome 11 respectively[10]. Recent studies showed that low miR-129-5p expression is associated with poor prognosis in breast cancer[11]. Mechanistically, miR-

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129-5p can attenuate irradiation-induced autophagy and decrease radioresistance of breast cancer cells by targeting hmgb1[12]. The downregulation of miR-129-5p is partly due to promoter hypermethylation[13]. However, whether other mechanism contributes to its downregulation is not clear. Enhancer of zeste homolog 2 (EZH2) is a subunit of the Polycomb repressor complex 2 (PRC2) that enhances DNA methylation by recruiting DNA methyl transferases[14]. Besides, EZH2 also acts as a histone methyl transferase that catalyzes tri-methylation of lysine 27 on histone 3 (H3K27me3), an epigenetic modification silencing gene transcription[15].

In this study, we firstly studied whether H3K27me3 modification is a mechanism of miR-129-5p downregulation in breast cancer and further investigated the functional role of miR-129-5p in EMT and multi-drug resistance (MDR) of the cancer cells.

Materials ad Methods

This study was approved by the Committee on Medical Ethics of the Affiliated Hospital of Hebei University of Engineering.

Cell Culture and Treatment

The human breast cancer cell line MCF-7 was obtained from American Type Culture Collection (Manassas, VA, USA). The Adriamycin (ADM)-resistant MCF-7/ADM cells were generated by a conventional stepwise method that gradually increases concentrations of ADM up to 100 nM over 8 months. The cancer cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 100 μg/mL penicillin, and 100 U/mL streptomycin at 37°C in 5% CO_2.

MiR-129-5p mimics and the scramble negative controls were purchased from Ribobio Co., Ltd. (Guangzhou, China). The lentiviral SOX4 expression vector pWPXL-SOX4 and EZH2 expression vector pCMVHA-hEZH2 expression vector were obtained from Addgene (Cambridge, MA, USA). The lentiviral particles for infection were prepared by co-transfection of the expression plasmids with the packaging constructs into HEK-293T cells. MCF-7 cells were transfected with pCMVHA-hEZH2 expression vector, 50 nM miR-129-5p mimics or the corresponding negative controls using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). MCF-7/ADM cells were transfected with 100 nM miR-129-5p mimics or the corresponding negative controls using Lipofectamine 2000 (Invitrogen). For SOX4 overexpression, MCF-7 cells were infected with pWPXL-SOX4 lentiviral particles in the presence of 8 μg/mL polybrene (Sigma-Aldrich, St. Louis, MO, USA).

QRT-PCR Analysis

Total RNA was extracted from the cell samples using TRIzol reagent (Invitrogen). Then, the first strand cDNA was reversely transcribed using the PrimeScript® RT reagent kit (TaKaRa, Dalian, Shandong, China). The sequence of the primers for SOX4 and EZH2 followed the design described in one previous study[16]. QRT-PCR was performed using SYBR® Premix Dimmer Eraser kit (TaKaRa) in an ABI Prism 7500 (Applied Biosystems, Foster City, CA, USA). GAPDH was used as the endogenous control.

To detect mature miR-129-5p, miR-129-1-3p and miR-129-2-3p expression, miRNA specific cDNA was firstly synthesized using the stem-loop primers and the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. Then, qRT-PCR was performed using the TaqMan MicroRNA Assay Kit (Applied Biosystems) to detect the relative miRNA expression. The $2^{-\Box\Box Ct}$ method was used to calculate relative mRNA and miRNA expression.

Western Blot Analysis

Total protein was extracted from the cell samples using RIPA buffer (Beyotime, Shanghai, China). The protein samples were denatured and then fractionated on 10% SDS-polyacrylamide gel. After that, the proteins were transferred to nitrocellulose membrane and subjected to primary antibody incubation. The primary antibodies used include anti-EZH2 (1:1000, #4905, Cell Signaling, Danvers, MA, USA), anti-SOX4 (1:5000, ab86809, Abcam), anti-E-cadherin (1:1000, #3195, Cell Signaling), anti-N-cadherin (1:1000, #13116, Cell Signaling), anti-Vimentin (1:1000, #5741, Cell Signaling) and anti-β-actin (1: 2000, ab8227, Abcam). After incubation with an appropriate secondary HRP conjugated antibody, the protein bands were detected using an ECL chromogenic substrate (Bio-Rad, Hercules, CA, USA). The gray scale was analyzed using Image J software.

Immunoprecipitation (IP)

MCF-7 cells were collected and then were lysed using ice-cold IP lysis buffer (Pierce, Rockford, IL, USA). The lysate was incubated

on ice with periodic mixing and then centrifuged at 13,000×g for 10 minutes at 4°C to remove the cell debris. Then, the lysate samples containing 500 μg proteins were immunoprecipitated with the agarose-immobilized antibody (8 μg of anti-SOX4, EZH2, H3K27me3 or isotype control antibodies respectively) overnight at 4°C. Then, the immune complexes were eluted from the agarose beads and analyzed by SDS-PAGE followed by immunoblot analysis.

Chromatin Immunoprecipitation (ChIP)

The ChIP assays were performed by using the Magna ChIP™ A/G Chromatin Immunoprecipitation Kit (Millipore, Temecula, CA, USA) according to manufacturer's instruction. In brief, the cells were fixed, lyzed and then fragmented by sonication into fragment sizes ranging from 200 to 1000 bp. Equal volumes of chromatin were immunoprecipitated with Dynal magnetic bead (Invitrogen, Carlsbad, CA, USA)-coupled antibody against EZH2 (#4905, Cell Signaling, Danvers, MA, USA), SOX4 (Ab86809, Abcam, Cambridge, MA, USA), H3K27me3 (ab6002, Abcam, Cambridge, MA, USA), or isotype IgG at 4°C overnight. The cross-links for the enriched and the input DNA were then reversed and the DNA was cleaned by RNase A (0.2 mg/mL) and proteinase K (2 μg/mL). The DNA samples were further purified and then used for qRT-PCR analysis by using a pair of primers (forward: 5'-GCTCTGGGGGAGTTTTTCTC-3'; reverse: 5'-CCTGCCTGTCCTATCCCTCT-3') amplified a 183 bp sequence upstream of transcription start of miR-129-2.

Fluorescence Microscopy

MCF-7 cells transfected with miR-129-5p mimics or the scramble negative controls were grown on coverslips. Then, the cells were fixed in methanol, permeabilized in 0.1% Triton X-100 and blocked with 1% BSA. The coverslips were then probed with primary antibodies against E-cadherin (#3195, Cell Signaling) and N-cadherin (#13116, Cell Signaling) at 4°C overnight. After the incubation, the coverslips were washed and further incubated with secondary Alexa Fluor®555-conjugated anti-rabbit IgG (#4413, Cell Signaling) and Alexa Fluor®488-conjugated anti-rabbit IgG (#4412, Cell Signaling) respectively for 1 hour at room temperature. Nuclei were stained using Prolong® Gold Antifade Reagent with DAPI (#8961, Cell Signaling). The images were captured using a fluorescence microscope.

Drug Sensitivity Assay

24 hours after transfection with miR-129-5p mimics, MCF-7/ADM cells were seeded in a 96-well plate. 24 hours later, the cells were treated with varying concentrations of ADM, Vincristine (VCR) or paclitaxel (PTX) for 48 hours. Then, cell viability was measured using a conventional MTT (Sigma-Aldrich, St. Louis, MO, USA) assay. Absorbance was recorded at 490 nm using a microplate reader. IC50 value was determined by creating dose-response curves.

Statistical Analysis

Data were presented in the form of means ± standard deviation. The group difference was compared by using the unpaired *t*-test. A two-sided *p*-value of < 0.05 was considered statistically significant.

Results

SOX4 and EZH2 Upregulation Result in miR-129-5p Downregulation in Breast Cancer Cells

MiR-129-5p is a tumor suppressive miRNA in breast cancer[12]. Previous studies demonstrated its expression is downregulated partly due to promoter hypermethylation[13,17]. In this study, we further studied whether EZH2 mediated H3K27me3 modification is a mechanism of miR-129-5p downregulation in breast cancer. MCF-7 cells were firstly transfected with EZH2 expression vector (Figure 1A-C). One recent study[16] showed that SOX4 can act as a co-repressor interacting with EZH2 and HDAC3 to suppress miRNA expression. Therefore, MCF-7 cells were also transfected with SOX4 expression vector (Figure 1D-F). QRT-PCR analysis confirmed that both enforced EZH2 and SOX4 expression resulted in suppressed miR-129-5p level in MCF-7 cells (Figure 1G-H).

MiR-129-5p is Decreased Partly due to EZH2 Mediated H3K27me3 Modification in the Promoter of miR-129-2

To further investigate the involvement of EZH2 mediated H3K27me3 modification in miR-129-5p suppression, coimmunoprecipitation (co-IP) was performed by using anti-SOX4, anti-EZH2 and anti-H3K27me3 in MCF-7 cell lysates. The results showed an interaction among SOX4, EZH2 and H3K27me3 modification (Figure 2A). In fact, miR-129-5p is transcribed from two genes, includ-

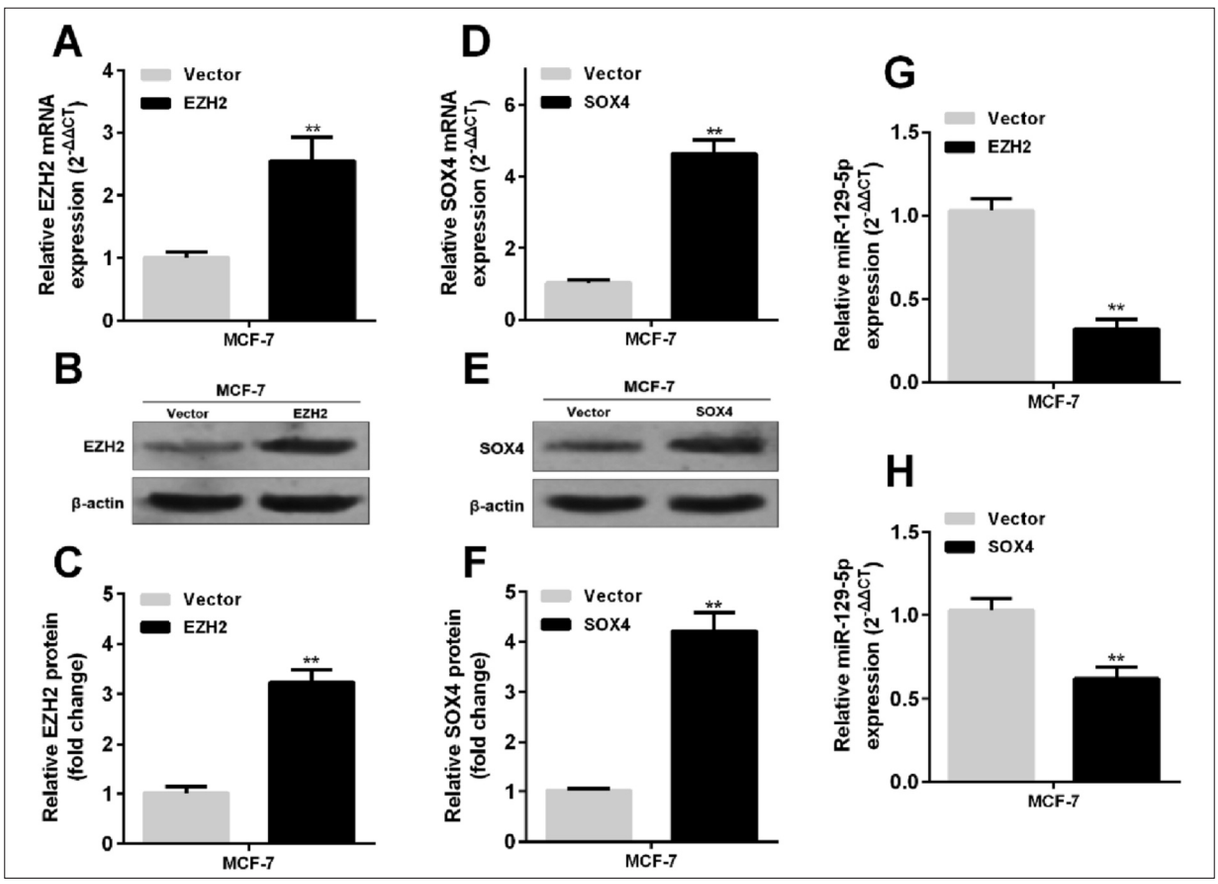

Figure 1. SOX4 and EZH2 upregulation lead to miR-129-5p downregulation in breast cancer cells. ***A,-F,*** QRT-PCR analysis of EZH2 mRNA ***(A)*** and SOX4 mRNA ***(D)***, images of Western blot of EZH2 ***(B)*** and SOX4 ***(E)*** and quantitation of the relative gray scale of EZH2 ***(C)*** and SOX4 ***(F)*** bands in MCF-7 cells after transfection of EZH2 expression vectors (pCMVHA-hEZH2) ***(A-C)*** or infected with SOX4 lentiviral particles (pWPXL-SOX4) or the corresponding negative control. ***G-H***, QRT-PCR of miR-129-5p expression in MCF-7 cells after transfection of pCMVHA-hEZH2 ***(G)*** or infected with pWPXL-SOX4 ***(H)***. $^{**}p < 0.01$.

ing miR-129-1 and miR-129-2 respectively (Figure 2B). To identity the exact host gene affected by EZH2 mediated H3K27me3 modification, qRT-PCR analysis was performed to detect miR-129-5p, miR-129-1-3p and miR-129-2-3p level after enforced SOX4 and EZH2 expression. The results showed that miR-129-5p and miR-129-2-3p were significantly downregulated after SOX4 and EZH2 overexpression (Figure 2C-D). However, miR-129-1-3p was almost unchanged (Figure 2C-D). Therefore, we hypothesized that EZH2 mediated H3K27me3 modification might be associated with dysregulated miR-129-2 transcription. Then, ChIP assay was performed to test this hypothesis. By using primers designed for the upstream of transcription start of miR-129-2, we found significant enrichment of SOX4, EZH2 and H3K27me3 in the region (Figure 2E). These results suggest that miR-129-5p is decreased partly due to EZH2 mediated H3K27me3 modification in the promoter of miR-129-2.

MiR-129-5p and SOX4 Are Reciprocally Regulated in Breast Cancer Cells

Previous studies[18,19] reported that miR-129 can decrease SOX4 expression by targeting the 3'UTR. The bioinformatics analysis showed that miR-129-5p has four possible targeting sites in the 3'UTR of SOX4 (Figure 3A). Following western blot analysis confirmed that MCF-7 cells transfected with miR-129-5p mimics had significantly suppressed SOX4 expression (Figure 3B). Therefore, we infer that there is a reciprocal regulation between miR-129-5p and SOX4 via the SOX4/EZH2 complex mediated H3K27me3 modification in breast cancer cells (Figure 3C).

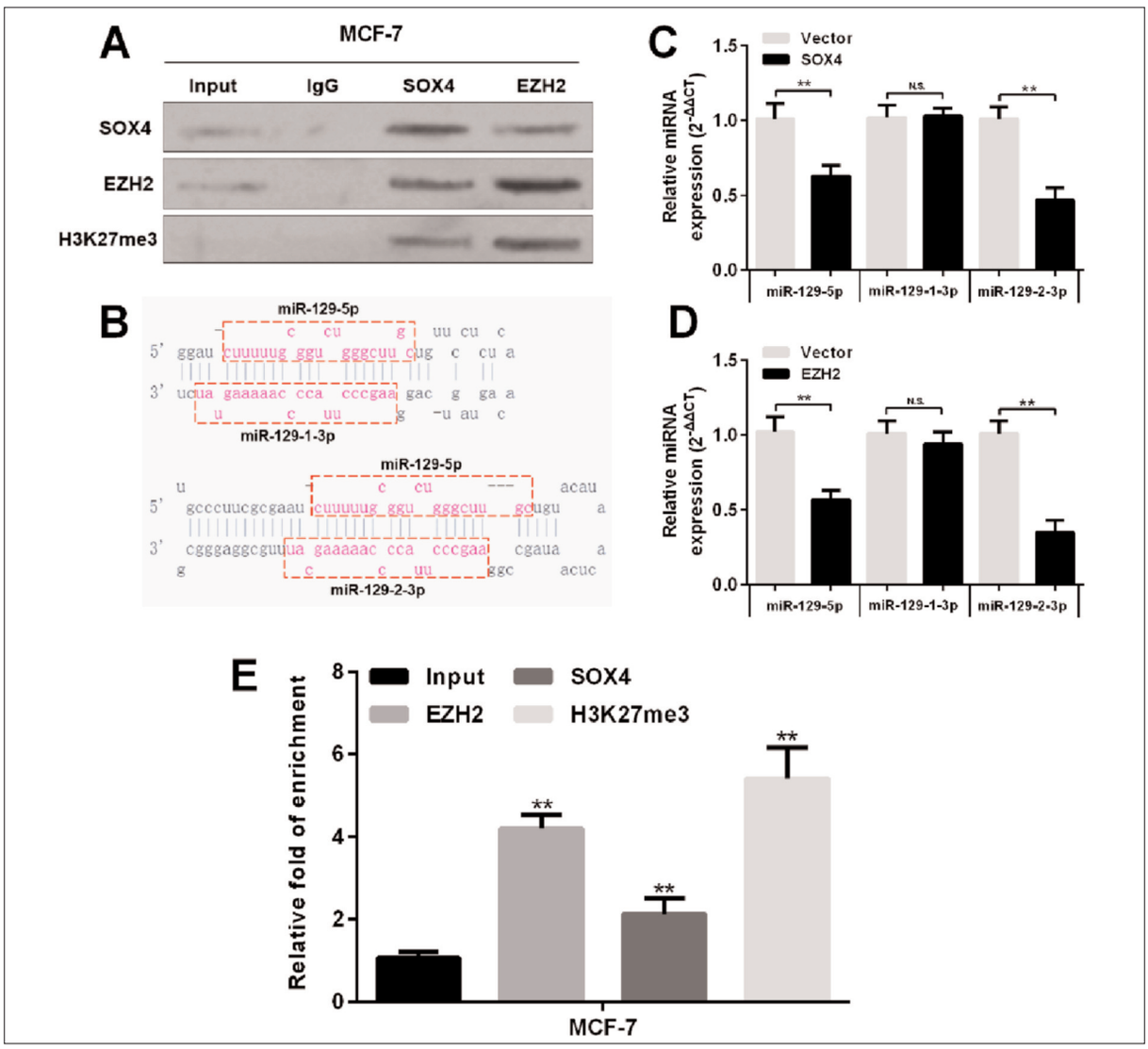

Figure 2. MiR-129-5p is decreased partly due to EZH2 mediated H3K27me3 modification in the promoter of miR-129-2. ***A,*** MCF-7 cell lysates were immunoprecipitated with SOX4, EZH2 or IgG control antibodies and immunoblots were probed for indicated antibodies including anti-SOX4, anti-EZH2 and anti-H3K27me3. ***B,*** Stem loop sequence of miR-129-1 and miR-129-2. ***C,-D,*** QRT-PCR analysis of miR-129-5p, miR-129-1-3p and miR-129-2-3p expression in MCF-7 cells after enforced expression of SOX4 ***(C)*** and EZH2 ***(D)***. ***E,*** Quantitative ChIP assay of the enrichment of SOX4, EZH2 and H3K27me3 on the proximal promoter region of miR-129-2 in MCF-7 cells. N.S. not significant; $^{**}p < 0.01$.

MiR-129-5p is an Important miRNA Modulating EMT and MDR of Breast Cancer Cells

SOX4 has been reported as a master control gene of EMT[20], which contributes to breast cancer progression[21,22]. Since we confirmed a feedback regulation between miR-129-5p and SOX4, we decided to further verify the role of miR-129-5p in EMT of breast cancer cells. QRT-PCR analysis showed that MCF-7/ADM cells had significantly decreased miR-129-5p expression (Figure 4A). MCF-7/ADM cells were transfected with miR-129-5p mimics (Figure 4B). Western blot analysis showed that MCF-7 cells with miR-129-5p overexpression had significantly increased E-cadherin and suppressed Vimentin expression (Figure 4C-D). MCF-7/ADM cells had a significantly lower expression of E-cadherin and a higher expression of N-cadherin and Vimentin than MCF-7 cells (Figure 4C). Enforced miR-129-5p expression also significantly increased E-cadherin and suppressed N-cadherin

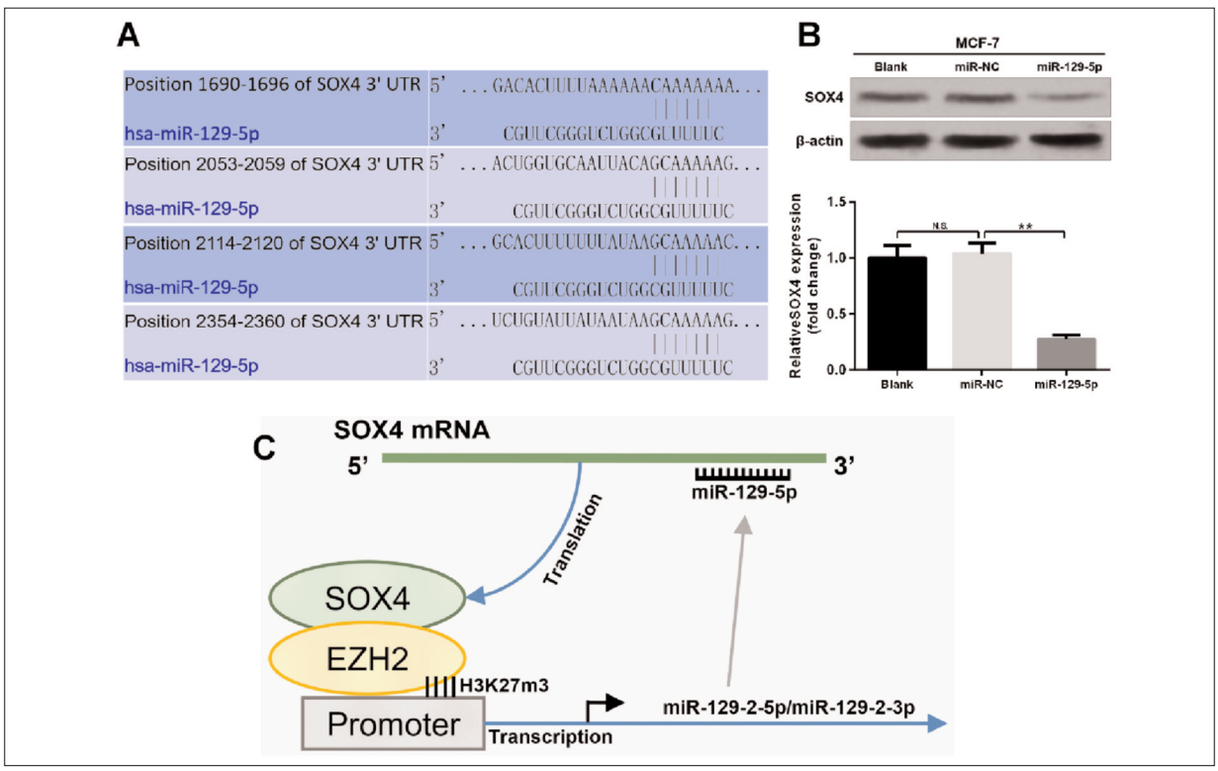

Figure 3. MiR-129-5p and SOX4 are reciprocally regulated in breast cancer cells. ***A,*** The predicted binding sites between miR-129-5p and the 3'UTR of SOX4. ***B,*** Images of western blot of SOX4 and quantitation of the relative gray scale of SOX4 bands in MCF-7 cells with or without transfection of miR-129-5p mimics. ***C,*** The schematic image of the reciprocal regulation between miR-129-5p and SOX4 via the SOX4/EZH2 mediated H3K27me3 modification. N.S. not significant; $^{**}p < 0.01$.

and Vimentin expression in MCF-7/ADM cells (Figure 4 C and E). Following immunofluorescent staining confirmed that miR-129-5p reversed the mesenchymal properties of MCF-7/ADM cells (Figure 4F). EMT has been considered as an important mechanism of increased MDR in breast cancer[23,24]. Then, we further investigated the regulative effect of miR-129-5p on MDR of breast cancer cells. Enforced miR-129-5p expression resulted in decreased P-gp expression, which is an important MDR related protein (Figure 4G). Following drug sensitivity assay showed that miR-129-5p substantially reduced IC50 of ADM, VCR and PTX in the MCF-7/ADM cells (Figure 4H).

Discussion

MiR-129-5p is a tumor suppressor in breast cancer[11]. Previous studies[13,17] reported that miR-129-2, which encodes miR-129-5p and miR-129-2-3p was epigenetically downregulated in breast cancer as a result of promoter methylation in gastric cancer and in breast cancer. Besides hypermethylation, EZH2 mediated H3K27me3 modification in the promoter region of miRNAs is also an important epigenetic regulation that silences miRNA expression in multiple types of cancers. For example, EZH2 mediated H3K27me3 modification can suppress miR-218 in non-small lung cancer cells[25], miR-31 in invasive esophageal cancer cells[16] and miR-200c in prostate cancer cells[26]. In breast cancer, miR-125b-1 is repressed by H3K9me3 or H3K27me3 in a different subtype of breast cancer cell lines[27]. In this study, we observed that EZH2 and SOX4 overexpression could decrease miR-129-5p levels in MCF-7 cells. Previous studies reported that SOX4 can enhance H3K27me3 by promoting the expression of EZH2[28] and forming a modification complex with EZH2 and HDAC3[16]. Therefore, we hypothesized that EZH2 mediated H3K27me3 modification might be a mechanism of miR-129-5p downregulation in breast cancer cells. By performing co-IP, we confirmed an interaction among SOX4,

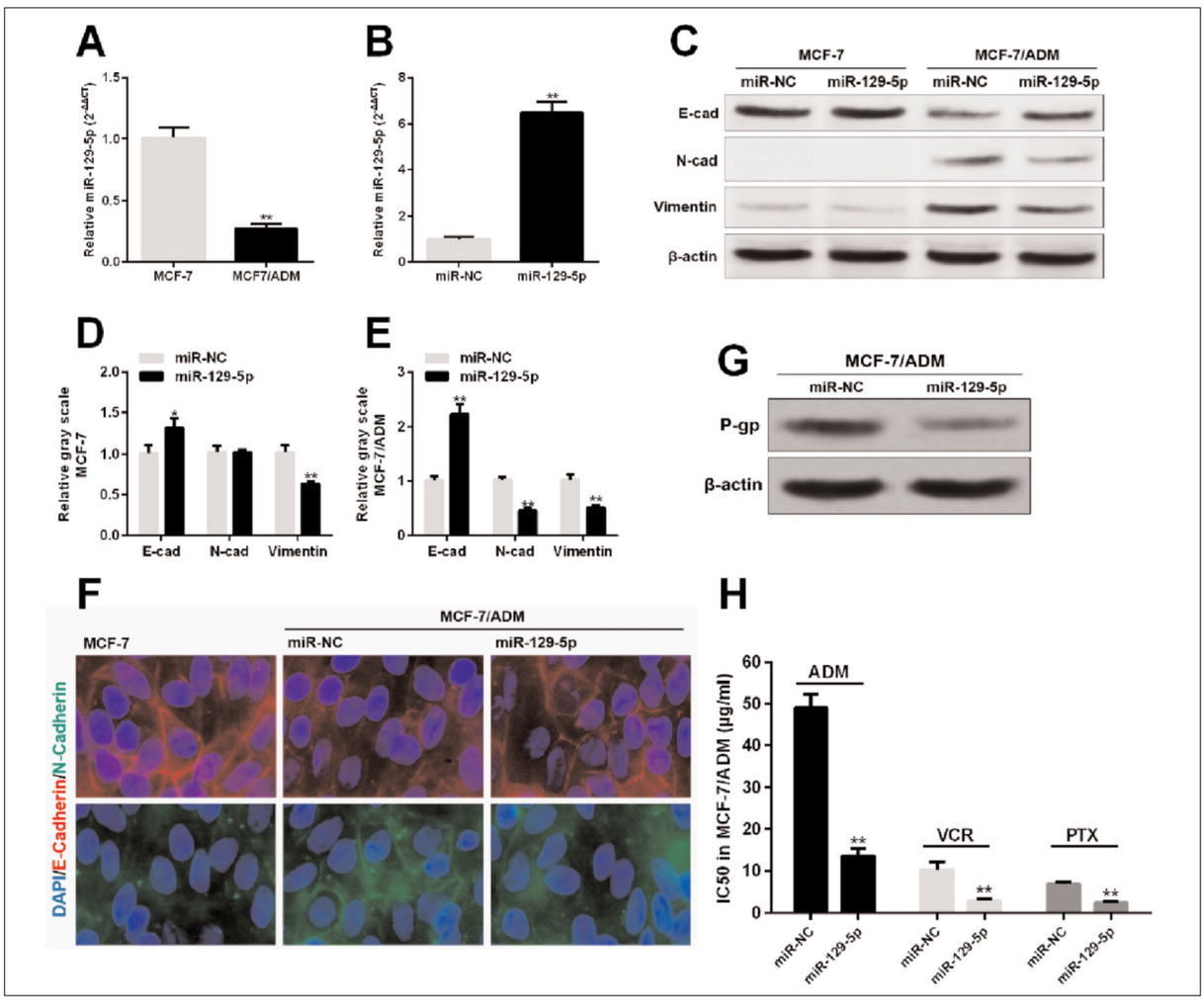

Figure 4. MiR-129-5p is an important miRNA modulating EMT and MDR of breast cancer cells. ***A,-B,*** QRT-PCR analysis of miR-129-5p expression in MCF-7 and MCF-7/ADM cells ***(A)*** and in MCF-7/ADM cells after transfection of miR-129-5p mimics or the negative control ***(B)***. ***C,-E,*** Western blot images ***(C)*** and quantitation of the relative gray scale ***(D-E)*** of E-cadherin (E-cad), N-cadherin (N-cad) and Vimentin expression in MCF-7 cells (D) and MCF-7/ADM cells ***(E)*** after transfection of miR-129-5p mimics or the negative control. F. Immunofluorescent staining (red: E-cadherin, green: N-cadherin, Blue: DAPI) in MCF-7 cells and in MCF-7/ADM cells after transfection of miR-129-5p mimics or the negative control. ***G,*** Western blot analysis of P-gp expression in MCF-7/ADM cells after transfection of miR-129-5p mimics or the negative control. H. ADM, VCR and PTX IC50 in MCF-7/ADM cells after transfection of miR-129-5p mimics or the negative control. $^{**}p < 0.01$.

EZH2 and H3K27me3 and the following ChIP assay showed significant enrichment of SOX4, EZH2 and H3K27me3 in the promoter region of miR-129-2. These results verified our hypothesis that miR-129-5p downregulation is partly due to EZH2 mediated H3K27me3 modification.

Previous studies reported that SOX4 is a direct target gene of miR-129-5p in peritoneal mesothelial cell[19] and is also a target gene of miR-129-5p and miR-129-2-3p in endometrial cancer cells[29], gastric cancer cells[17], esophageal carcinoma cells[30] and in hepatocellular cancer cells[31]. Our Western blot analysis also confirmed that miR-129-5p mimics can decrease SOX4 expression in MCF-7 cells. Therefore, we infer that there might be a feedback regulation between miR-129-5p and SOX4 in breast cancer cells. High SOX4 expression serves as an unfavorable prognostic biomarker in breast cancer patients[22]. Mechanistically, SOX4 acts as a master of EMT programs, which facilitates progression of metastatic breast cancer[20,28]. As a miRNA with regulative effect on SOX4 expression, we also found that miR-129-5p can suppress EMT of the MCF-7 cells.

Previous studies reported that enhanced EMT is not only a mechanism contributing to higher invasive potential in breast cancer cells, but also a cause of increased MDR[23,32]. Multiple miRNAs can regulate drug sensitivity of breast cancer cells via modulating EMT. For example, Forced miR-489 expression in MCF-7/ADM cells can both inhibit Smad3 expression and Smad3 related EMT properties, leading to reduced chemoresistance[33]. MiR-644a can directly target the transcriptional co-repressor C-Terminal Binding Protein 1 (CTBP1), which subsequently leads to upregulation of p53[34]. The miR-644a/CTBP1/p53 axis thereby suppresses drug resistance by simultaneous inhibition of cell survival and EMT in breast cancer cells[34]. MiR-129-5p was also reported as a modulator of MDR in gastric cancer cells. Restoration of miR-129-5p can reduce the chemoresistance of SGC7901/VCR and SGC7901/ADR cells via decreasing the expression of MDR related ABC transporters, including ABCB1, ABCC5 and ABCG1[6]. In this study, we further investigated the role of miR-129-5p in MDR of MCF-7/ADM cells. The results showed that miR-129-5p reversed the mesenchymal properties of MCF-7/ADM cells. In addition, enforced miR-129-5p expression resulted in decreased P-gp expression and reduced IC50 of ADM, VCR and PTX in MCF-7/ADM cells.

Conclusions

There is a reciprocal regulation between miR-129-5p and SOX4 via the SOX4/EZH2 complex mediated H3K27me3 modification in breast cancer cells. MiR-129-5p is an important miRNA modulating EMT and MDR in breast cancer cells.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Zhang Z, Zhang B, Li W, Fu L, Fu L, Zhu Z, Dong JT. Epigenetic Silencing of miR-203 Upregulates SNAI2 and Contributes to the Invasiveness of Malignant Breast Cancer Cells. Genes Cancer 2011; 2: 782-791.
2) Zhang N, Wang AY, Wang XK, Sun XM, Xue HZ. GAS5 is downregulated in gastric cancer cells by promoter hypermethylation and regulates adriamycin sensitivity. Eur Rev Med Pharmacol Sci 2016; 20: 3199-3205.
3) Alberti C. Prostate cancer cell both microRNA aberrant expression-mediated autophagy and radiation therapy resistance with short cross-reference to epigenetic/genetic mechanism implications. Eur Rev Med Pharmacol Sci 2016; 20: 1001-1002.
4) Vrba L, Munoz-Rodriguez JL, Stampfer MR, Futscher BW. miRNA gene promoters are frequent targets of aberrant DNA methylation in human breast cancer. PLoS One 2013; 8: e54398.
5) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
6) Wu Q, Yang Z, Xia L, Nie Y, Wu K, Shi Y, Fan D. Methylation of miR-129-5p CpG island modulates multi-drug resistance in gastric cancer by targeting ABC transporters. Oncotarget 2014; 5: 11552-11563.
7) Xiao C, Wu CH, Hu HZ. LncRNA UCA1 promotes epithelial-mesenchymal transition (EMT) of breast cancer cells via enhancing Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2016; 20: 2819-2824.
8) Ye XM, Zhu HY, Bai WD, Wang T, Wang L, Chen Y, Yang AG, Jia LT. Epigenetic silencing of miR-375 induces trastuzumab resistance in HER2-positive breast cancer by targeting IGF1R. BMC Cancer 2014; 14: 134.
9) He DX, Gu XT, Li YR, Jiang L, Jin J, Ma X. Methylation-regulated miR-149 modulates chemoresistance by targeting GlcNAc N-deacetylase/N-sulfotransferase-1 in human breast cancer. FEBS J 2014; 281: 4718-4730.
10) Yu X, Song H, Xia T, Han S, Xiao B, Luo L, Xi Y, Guo J. Growth inhibitory effects of three miR-129 family members on gastric cancer. Gene 2013; 532: 87-93.
11) Yu Y, Zhao Y, Sun XH, Ge J, Zhang B, Wang X, Cao XC. Down-regulation of miR-129-5p via the Twist1-Snail feedback loop stimulates the epithelial-mesenchymal transition and is associated with poor prognosis in breast cancer. Oncotarget 2015.
12) Luo J, Chen J, He L. mir-129-5p Attenuates Irradiation-Induced Autophagy and Decreases Radioresistance of Breast Cancer Cells by Targeting HMGB1. Med Sci Monit 2015; 21: 4122-4129.
13) Tang X, Tang J, Liu X, Zeng L, Cheng C, Luo Y, Li L, Qin SL, Sang Y, Deng LM, Lv XB. Downregulation of miR-129-2 by promoter hypermethylation regulates breast cancer cell proliferation and apoptosis. Oncol Rep 2016; 35: 2963-2969.
14) Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM, Bollen M, Esteller M, Di Croce L, De Launoit Y, Fuks F. The Polycomb group protein EZH2 directly controls DNA methylation. Nature 2006; 439: 871-874.

15) Cao R, Zhang Y. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr Opin Genet Dev 2004; 14: 155-164.

16) Koumangoye RB, Andl T, Taubenslag KJ, Zilberman ST, Taylor CJ, Loomans HA, Andl CD. SOX4 interacts with EZH2 and HDAC3 to suppress microRNA-31 in invasive esophageal cancer cells. Mol Cancer 2015; 14: 24.

17) Shen R, Pan S, Qi S, Lin X, Cheng S. Epigenetic repression of microRNA-129-2 leads to overexpression of SOX4 in gastric cancer. Biochem Biophys Res Commun 2010; 394: 1047-1052.

18) Dyrskjot L, Ostenfeld MS, Bramsen JB, Silahtaroglu AN, Lamy P, Ramanathan R, Fristrup N, Jensen JL, Andersen CL, Zieger K, Kauppinen S, Ulhoi BP, Kjems J, Borre M, Orntoft TF. Genomic profiling of microRNAs in bladder cancer: miR-129 is associated with poor outcome and promotes cell death in vitro. Cancer Res 2009; 69: 4851-4860.

19) Xiao L, Zhou X, Liu F, Hu C, Zhu X, Luo Y, Wang M, Xu X, Yang S, Kanwar YS, Sun L. MicroRNA-129-5p modulates epithelial-to-mesenchymal transition by targeting SIP1 and SOX4 during peritoneal dialysis. Lab Invest 2015; 95: 817-832.

20) Parvani JG, Schiemann WP. Sox4, EMT programs, and the metastatic progression of breast cancers: mastering the masters of EMT. Breast Cancer Res 2013; 15: R72.

21) Zhang J, Liang Q, Lei Y, Yao M, Li L, Gao X, Feng J, Zhang Y, Gao H, Liu DX, Lu J, Huang B. SOX4 induces epithelial-mesenchymal transition and contributes to breast cancer progression. Cancer Res 2012; 72: 4597-4608.

22) Song GD, Sun Y, Shen H, Li W. SOX4 overexpression is a novel biomarker of malignant status and poor prognosis in breast cancer patients. Tumour Biol 2015; 36: 4167-4173.

23) Mallini P, Lennard T, Kirby J, Meeson A. Epithelial-to-mesenchymal transition: what is the impact on breast cancer stem cells and drug resistance. Cancer Treat Rev 2014; 40: 341-348.

24) Saxena M, Stephens MA, Pathak H, Rangarajan A. Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. Cell Death Dis 2011; 2: e179.

25) Wang B, Liu Y, Luo F, Xu Y, Qin Y, Lu X, Xu W, Shi L, Liu Q, Xiang Q. Epigenetic silencing of microRNA-218 via EZH2-mediated H3K27 trimethylation is involved in malignant transformation of HBE cells induced by cigarette smoke extract. Arch Toxicol 2016; 90: 449-461.

26) Tao T, Liu D, Liu C, Xu B, Chen S, Yin Y, Ang L, Huang Y, Zhang X, Chen M. Autoregulatory feedback loop of EZH2/miR-200c/E2F3 as a driving force for prostate cancer development. Biochim Biophys Acta 2014; 1839: 858-865.

27) Cisneros-Soberanis F, Andonegui MA, Herrera LA. miR-125b-1 is repressed by histone modifications in breast cancer cell lines. Springerplus 2016; 5: 959.

28) Tiwari N, Tiwari VK, Waldmeier L, Balwierz PJ, Arnold P, Pachkov M, Meyer-Schaller N, Schubeler D, Van Nimwegen E, Christofori G. Sox4 is a master regulator of epithelial-mesenchymal transition by controlling Ezh2 expression and epigenetic reprogramming. Cancer Cell 2013; 23: 768-783.

29) Huang YW, Liu JC, Deatherage DE, Luo J, Mutch DG, Goodfellow PJ, Miller DS, Huang TH. Epigenetic repression of microRNA-129-2 leads to overexpression of SOX4 oncogene in endometrial cancer. Cancer Res 2009; 69: 9038-9046.

30) Kang M, Li Y, Liu W, Wang R, Tang A, Hao H, Liu Z, Ou H. miR-129-2 suppresses proliferation and migration of esophageal carcinoma cells through downregulation of SOX4 expression. Int J Mol Med 2013; 32: 51-58.

31) Chen X, Zhang L, Zhang T, Hao M, Zhang X, Zhang J, Xie Q, Wang Y, Guo M, Zhuang H, Lu F. Methylation-mediated repression of microRNA 129-2 enhances oncogenic SOX4 expression in HCC. Liver Int 2013; 33: 476-486.

32) Li QQ, Xu JD, Wang WJ, Cao XX, Chen Q, Tang F, Chen ZQ, Liu XP, Xu ZD. Twist1-mediated adriamycin-induced epithelial-mesenchymal transition relates to multidrug resistance and invasive potential in breast cancer cells. Clin Cancer Res 2009; 15: 2657-2665.

33) Jiang L, He D, Yang D, Chen Z, Pan Q, Mao A, Cai Y, Li X, Xing H, Shi M, Chen Y, Bruce IC, Wang T, Jin L, Qi X, Hua D, Jin J, Ma X. MiR-489 regulates chemoresistance in breast cancer via epithelial mesenchymal transition pathway. FEBS Lett 2014; 588: 2009-2015.

34) Raza U, Saatci O, Uhlmann S, Ansari SA, Eyupoglu E, Yurdusev E, Mutlu M, Ersan PG, Altundag MK, Zhang JD, Dogan HT, Guler G, Sahin O. The miR-644a/CTBP1/p53 axis suppresses drug resistance by simultaneous inhibition of cell survival and epithelial-mesenchymal transition in breast cancer. Oncotarget 2016 Jul 8. doi: 10.18632/oncotarget.10489. [Epub ahead of print].