

MicroRNA-1297 downregulation inhibits breast cancer cell epithelial-mesenchymal transition and proliferation in a FA2H-dependent manner

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Abstract. Breast cancer (BC) is one of the most common malignant tumors among women worldwide. MicroRNAs (miRs) may be involved in several types of human cancer, including gastric, liver, lung and breast cancer. The aim of the present study was to investigate the effect of miR-1297 on MDA-MB-231 cell epithelial-mesenchymal transition (EMT) and proliferation, and the underlying molecular mechanisms. MDA-MB-231 cells were transfected with miR-1297 inhibitor or inhibitor control for 48 h. Subsequently, MTT and flow cytometry assays indicated that miR-1297 inhibitor significantly decreased cell proliferation and induced apoptosis compared with the inhibitor control group. In addition, reverse transcription-quantitative PCR and western blotting suggested that miR-1297 inhibitor suppressed EMT in MDA-MB-231 cells compared with the inhibitor control group. TargetScan bioinformatics analysis and a dual-luciferase reporter gene assay were performed, which predicted that miR-1297 directly targeted fatty acid 2-hydroxylase (FA2H). Furthermore, MDA-MB-231 cells were transfected with control-plasmid or FA2H-plasmid for 48 h. The results demonstrated that FA2H overexpression decreased MDA-MB-231 cell proliferation and increased apoptosis compared with the control-plasmid group. Additionally, FA2H-plasmid increased E-cadherin expression levels, and reduced N-cadherin and matrix metalloproteinase 9 expression levels at both the protein and mRNA level compared with control-plasmid. Finally, MDA-MB-231 cells were transfected with control-small interfering (si)

RNA, FA2H-siRNA, inhibitor control, miR-1297 inhibitor, miR-1297 inhibitor + control siRNA or miR-1297 inhibitor + FA2H-siRNA, and the results suggested that the biological effects of miR-1297 inhibitor were reversed by co-transfection with FA2H siRNA. In conclusion, the present study indicated that miR-1297/FA2H might serve as a novel potential biomarker and therapeutic target for BC.

Introduction

Breast cancer (BC) is one of the most common types of cancer in women worldwide and a number of patients exhibit an increased risk of metastasis and recurrence (1-3). It is estimated that ~50% of BC cases and 60% of BC-related deaths occur in developing countries, with the majority of deaths caused by cancer metastasis (4). With the advancement and development of medical standards, the mortality rate of patients with BC has steadily decreased in the past few decades. However, although breast cancer death rates have dropped 34% since 1990, not all segments of the population have benefited from this decrease (5).

MicroRNAs (miRNAs/miRs) are a group of endogenous, non-coding, single-stranded RNAs, 18-24 nucleotides in length, which mediate downstream gene expression at the post-transcriptional level (6-8). miRNAs are involved in several biological processes, and their expression and functions are associated with numerous diseases, such as cancer and diseases of the digestive, nervous and cardiovascular systems (9). An increasing number of studies have suggested that miRNAs are abnormally expressed in multiple developmental processes of BC (10,11). miR-1297 is a novel cancer-related miRNA that serves a crucial role in the pathogenesis of human cancer (12-16). Dysregulation of miR-1297 has been detected in various types of human cancer (17-20). For example, Chen *et al* (17) reported that miR-1297 expression was decreased in pancreatic adenocarcinoma tissues. Gao *et al* (18) suggested that miR-1297 expression was significantly lower in gastric cancer tissue samples compared with adjacent healthy tissue samples. Moreover, increases in miR-1297 expression levels were observed in laryngeal squamous cell carcinoma (19) and BC (20) tissues. miR-1297 can exist as an oncogene or a tumor suppressor

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gene in tumor cells (15,20-22). Wang *et al* (15) demonstrated that miR-1297 suppressed glioma cell proliferation via targeting high mobility group A1. In addition, Liu *et al* (21) demonstrated that miR-1297 upregulation attenuated cell proliferation via modulating enhancer of zeste homolog 2, whereas Liang *et al* (22) revealed that miR-1297 was involved in the development of oral squamous cell carcinoma via regulating phosphatase and tensin homolog (PTEN). Furthermore, a previous study suggested that miR-1297 was increased in BC tissues and cell lines, and promoted BC cell proliferation (20). However, the effect of miR-1297 on BC cells is not completely understood. The present study aimed to investigate the effects of miR-1297 on BC cell epithelial-mesenchymal transition (EMT) and proliferation, and the underlying molecular mechanisms.

As a hydroxy fatty acid enzyme, fatty acid 2-hydroxylase (FA2H) promotes 2-hydroxylation of fatty acid N-acyl chain (23). It has been reported that 2-hydroxyceramide and FA2H, which are expressed in several tissues (24,25), participate in multiple cell signaling pathways, such as the AMPK and mTOR/p70 ribosomal protein S6 kinase 1 (S6K1)/glioma-associated oncogene homolog 1 pathways (26). FA2H also serves a crucial role in the occurrence and development of tumors (26). FA2H downregulation has been observed in triple negative breast cancer tissues and cell lines (27). FA2H expression levels were lower in gastric tumor tissues compared with healthy control tissues (26). Moreover, another study revealed that FA2H levels were higher in adenocarcinoma compared with squamous and neuroendocrine carcinoma (28). Previous studies have indicated that FA2H may affect cell cycle and migration, enhance the sensitivity of tumor cells to drugs and regulate drug resistance of tumor cells (26-28). In terms of drug resistance, FA2H may increase the therapeutic effect via enhancing the sensitivity of tumor cells to drugs (26,29). Accumulating evidence has suggested that FA2H exerts its role in promoting drug sensitivity via regulating endocytosis and exocytosis of drugs via the cell membrane (29). Additionally, it has been reported that FA2H is associated with shorter tumor-free survival in triple-negative BC (27). However, the specific roles of FA2H and 2-hydroxy fatty acids, as components of the metabolism, in regulating tumors and their underlying mechanisms are not completely understood.

The current study aimed to investigate the effect of miR-1297 and FA2H on breast cancer cell EMT and proliferation, and the underlying molecular mechanisms.

Materials and methods

Cell culture and transfection. MDA-MB-231 cells were cultured in DMEM (HyClone; Cytiva) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂. Subsequently, MDA-MB-231 cells (5x10⁴ cells/well; 24-well plates) were transfected with 100 nM inhibitor control (cat. no. MIH000000; Applied Biological Materials, Inc.), 100 nM miR-1297 inhibitor (cat. no. MIH01244; Applied Biological Materials, Inc.), 1 µg control-plasmid (cat. no. sc-437275; Santa Cruz Biotechnology, Inc.), 1 µg FA2H-plasmid (cat. no. sc-413143-ACT; Santa Cruz

Biotechnology, Inc.), 0.2 µM control-small interfering (si)RNA (cat. no. sc-36869; Santa Cruz Biotechnology, Inc.), 0.2 µM FA2H-siRNA (cat. no. sc-93418; Santa Cruz Biotechnology, Inc.), miR-1297 inhibitor + control-siRNA or miR-1297 inhibitor + FA2H-siRNA using Polyplus transfection reagent (Polyplus-transfection SA) according to the manufacturer's protocol. At 48 h post-transfection, cells were used for subsequent experiments.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from MDA-MB-231 cells using TRIzol® reagent (Takara Bio, Inc.) according to the manufacturer's protocol. The concentration of the RNA was detected using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA using the HiScript II 1st Strand cDNA Synthesis kit (Vazyme Biotech Co., Ltd.). The RT temperature conditions were as follows: 70°C for 5 min, 37°C for 5 min and 42°C for 60 min. Subsequently, qPCR was performed using a SYBR Green PCR kit (Vazyme Biotech Co., Ltd.). The following thermocycling conditions were used for qPCR: Initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 55°C for 40 sec and extension at 72°C for 34 sec. The following primers were used for qPCR: GAPDH, Forward, 5'-ATTCCATGGCACCGTCAAGGCTGA-3' and reverse: 5'-TTC TCCATGGTGGTGAAGACGCCA-3'; U6, forward: 5'-GCTTCGGCAGCACATATACTAAAAT-3' and reverse: 5'-CGCTTCACGAATTTGCGTGTGCAT-3'; N-cadherin, forward: 5'-TTTGATGGAGGTCTCCTAACACC-3' and reverse: 5'-ACGTTTAACACGTTGGAAATGTG-3'; E-cadherin, forward: 5'-CGAGAGCTACACGTTACGG-3' and reverse: 5'-GGGTGTCGAGGGGAAAATAGG-3'; matrix metalloproteinase (MMP)9, forward: 5'-GAACCAATCTCACCGACAGG-3' and reverse: 5'-CCACAACCTCGTCATCGTCG-3'; FA2H, forward: 5'-AGTACTATGTGGGCGAACTGC-3' and reverse: 5'-CAATAGCAGCATCTGTCTTCTGA-3'; miR-1297, forward: 5'-ACACTCCAGCTGGGTCCTTCATTCCA-3' and reverse: 5'-GTGCAGGGTCCGAGGT-3'. miRNA and mRNA expression levels were quantified using the 2^{-ΔΔC_q} method (30) and normalized to the internal reference genes U6 and GAPDH, respectively.

Western blotting. Total protein was extracted from cells using RIPA buffer (Beyotime Institute of Biotechnology). Total protein was quantified using a bicinchoninic acid assay kit (Pierce; Thermo Fisher Scientific, Inc.). Equal amounts of protein (40 µg per lane) were separated via 12% SDS-PAGE for 40 min and transferred onto PVDF membranes (EMD Millipore), which were blocked with 5% non-fat milk for 1.5 h at room temperature. Subsequently, the membranes were incubated at 4°C overnight with primary antibodies targeted against: E-cadherin (cat. no. ab15148; 1:1,000; Abcam), N-cadherin (cat. no. ab76057; 1:1,000; Abcam), MMP9 (cat. no. ab38898; 1:1,000; Abcam), FA2H (cat. no. ab128917; 1:1,000; Abcam) and GAPDH (cat. no. ab9485; 1:1,000; Abcam). The following day, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:2,000; cat. no. 7074; Cell Signaling Technology, Inc.) for 2 h at room temperature. Protein bands were visualized using the

enhanced chemiluminescence method (Cytiva). GAPDH was used as the loading control.

Flow cytometry assay. Cell apoptosis was assessed using the Annexin-V/PI Apoptosis Detection kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. Briefly, cells were seeded (2×10^5 cells/well) into 6-well plates and cultured at 37°C overnight. Following transfection, cells were harvested, centrifuged at $1,000 \times g$ at 4°C for 5 min, and the cell pellet was resuspended in $100 \mu\text{l}$ FITC-binding buffer. Subsequently, the cell suspension was incubated with $5 \mu\text{l}$ ready-to-use Annexin V-FITC (BD Bioscience) and $5 \mu\text{l}$ PI at room temperature in the dark for 30 min. Cell apoptosis (late or early + late apoptosis) was assessed via flow cytometry using a BD FACSCalibur flow cytometer (BD Biosciences). Data were analyzed using CellQuest™ version 5.1 software (BD Biosciences).

Dual luciferase reporter assay. Bioinformatics analysis was performed using TargetScan 7.2 (http://www.targetscan.org/vert_72/). And the results showed the potential binding sites between miR-1297 and the 3'-UTR of FA2H. The wild-type (WT) or mutant (MUT) 3'-untranslated regions (3'-UTRs) of FA2H were cloned into the pmirGLO vector (Promega Corporation). The recombinant plasmids were acquired using an EndoFree Plasmid Maxi kit (Vazyme Biotech Co., Ltd.). Subsequently, 293T cells (5×10^4 cells/well; American Type Culture Collection) were seeded into 24-well plates and co-transfected with 50 nM miR-1297 mimic (cat. no. MCH01244; Applied Biological Materials, Inc.) or 50 nM mimic control (cat. no. MCH00000; Applied Biological Materials, Inc.) and 1 ng MUT FA2H 3'UTR or 1 ng WT FA2H 3'UTR using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 48 h. The pRL-TK plasmid (Promega Corporation) containing the *Renilla* luciferase gene was used as an internal control. At 48 h post-transfection, and *Renilla* luciferase activities were detected using the Dual-Luciferase Reporter Assay System (Promega Corporation). Firefly luciferase activity was normalized to *Renilla* luciferase activity. Besides, 293T cells were transfected with miR-1297 mimic or mimic control for 48 h, and the transfection efficiency was confirmed using qRT-PCR.

MTT assay. Cell proliferation was assessed using an MTT assay. Briefly, MDA-MB-231 cells were plated into a 96-well plate (5×10^4 cells per well) and incubated at 37°C for 24, 48 or 72 h. Subsequently, $20 \mu\text{l}$ MTT reagent (5 mg/ml; Sigma-Aldrich; Merck KGaA) was added to each well at 37°C for 4 h. In total, $150 \mu\text{l}$ DMSO (Beyotime Institute of Biotechnology) was used to dissolve the purple formazan. The absorbance was measured at a wavelength of 570 nm using a multifunctional plate reader (BD Biosciences) according to the assay manufacturer's instructions.

Statistical analysis. Statistical analyses were performed using GraphPad Prism software (version 6.0; GraphPad Software Inc.). Comparisons among groups were analyzed using the unpaired Student's t-test or one-way ANOVA followed by Tukey's post hoc test. Data are presented as the

mean \pm SD from at least three independent experiments. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of miR-1297 knockdown on MDA-MB-231 cell proliferation and apoptosis. To investigate the biological effects of miR-1297 on BC, MDA-MB-231 cells were transfected with inhibitor control or miR-1297 inhibitor for 48 h. Compared with the inhibitor control group, the RT-qPCR results indicated that miR-1297 inhibitor significantly decreased miR-1297 expression in MDA-MB-231 cells (Fig. 1A). In addition, the MTT assay results suggested that miR-1297 inhibitor decreased MDA-MB-231 cell proliferation at 24, 48 and 72 h compared with the inhibitor control group (Fig. 1B). Furthermore, the flow cytometry results indicated that miR-1297 inhibitor significantly increased MDA-MB-231 cell apoptosis compared with the inhibitor control group (Fig. 1C and D).

Effects of miR-1297 knockdown on MDA-MB-231 cell EMT. Subsequently, western blotting and RT-qPCR were performed to detect the expression levels of EMT indicators, an epithelial cell marker, E-cadherin, and two interstitial cell markers, N-cadherin and MMP9. The results indicated that miR-1297 inhibitor upregulated E-cadherin expression levels, and decreased N-cadherin and MMP9 expression levels at the protein and mRNA levels compared with the inhibitor control group (Fig. 2A-D).

miR-1297 directly targets FA2H. To identify the mechanisms underlying FA2H, bioinformatics analysis was performed using TargetScan. The results indicated that FA2H might be a potential downstream target gene of miR-1297 (Fig. 3A). Subsequently, the association between FA2H and miR-1297 was assessed by performing a dual luciferase activity assay. The results suggested that miR-1297 mimic significantly inhibited the luciferase activity of the WT FA2H 3'UTR reporter plasmid compared with mimic control. However, miR-1297 mimic had no significant effect on the luciferase activity of the MUT FA2H 3'UTR reporter plasmid compared with mimic control (Fig. 3B). Moreover, compared with the mimic control group, miR-1297 mimic significantly increased miR-1297 expression in 293T cells (Fig. 3C). The results indicated that FA2H was a direct target of miR-1297.

Effects of FA2H overexpression on MDA-MB-231 cell proliferation and apoptosis. To further investigate the biological effects of FA2H on BC, MDA-MB-231 cells were transfected with control-plasmid or FA2H-plasmid for 48 h. Compared with the control-plasmid group, FA2H overexpression increased FA2H mRNA and protein expression levels in MDA-MB-231 cells (Fig. 4A and B). Furthermore, compared with the control-plasmid group, FA2H overexpression decreased MDA-MB-231 cell proliferation at 24, 48 and 72 h (Fig. 4C). By contrast, FA2H overexpression significantly enhanced cell apoptosis compared with the control-plasmid group (Fig. 4D and E).

Effects of FA2H overexpression on MDA-MB-231 cell EMT. Furthermore, the expression levels of EMT indicators,

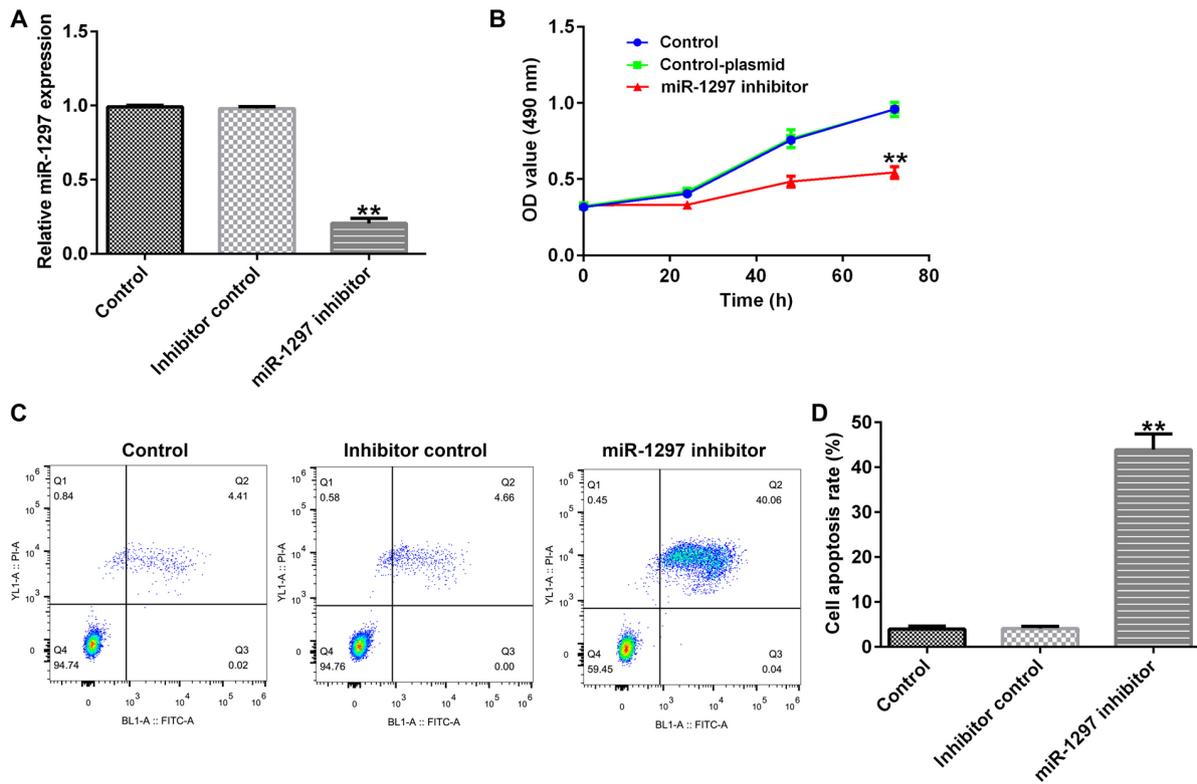


Figure 1. miR-1297 knockdown inhibits MDA-MB-231 cell proliferation. MDA-MB-231 cells were transfected with inhibitor control or miR-1297 inhibitor for 48 h. (A) miR-1297 expression levels were measured via reverse transcription-quantitative PCR. (B) MDA-MB-231 cell proliferation at 24, 48 and 72 h was measured by performing an MTT assay. Cell apoptosis was (C) determined via flow cytometry and (D) quantified. miR, microRNA; OD, optical density. ** $P < 0.01$ vs. inhibitor control group.

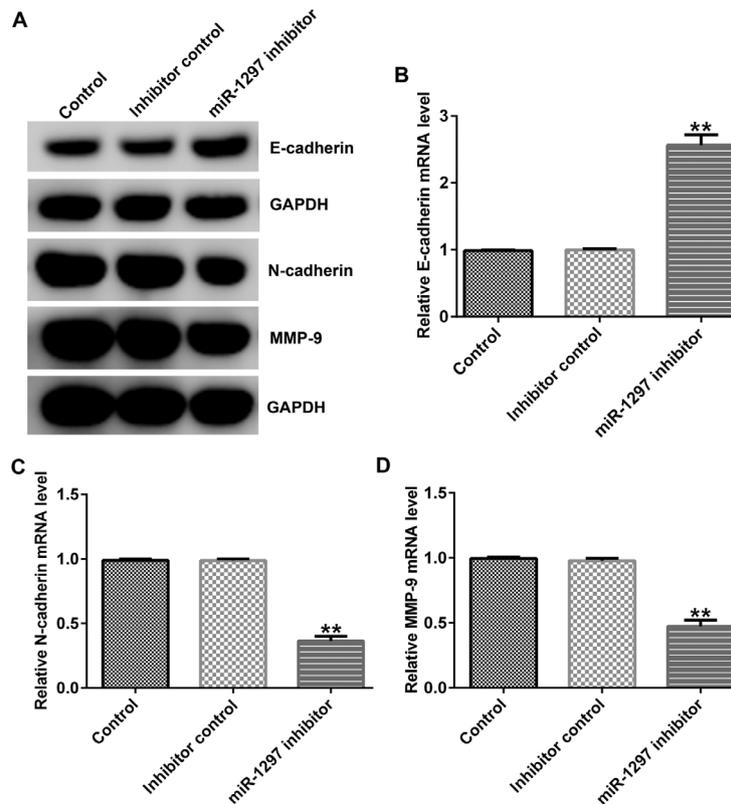


Figure 2. miR-1297 knockdown inhibits EMT. MDA-MB-231 cells were transfected with inhibitor control or miR-1297 inhibitor for 48 h. (A) Protein expression levels of E-cadherin, N-cadherin and MMP-9 were determined via western blotting. mRNA expression levels of (B) E-cadherin, (C) N-cadherin and (D) MMP-9 were determined via reverse transcription-quantitative PCR. miR, microRNA; EMT, epithelial-mesenchymal transition; MMP-9, matrix metalloproteinase-9. ** $P < 0.01$ vs. inhibitor control group.

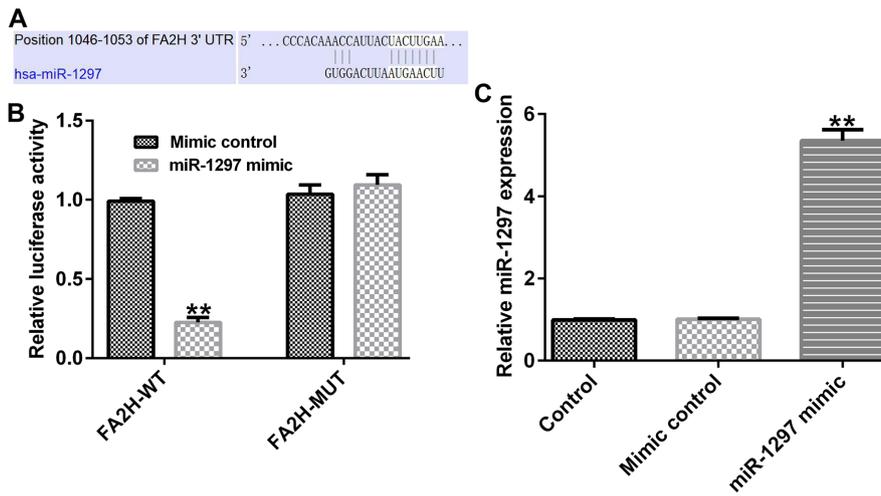


Figure 3. FA2H is directly targeted by miR-1297. (A) FA2H was predicted as a potential target of miR-1297 via TargetScan. (B) The dual luciferase reporter assay was performed to assess the association between miR-1297 and FA2H. (C) Transfection efficiency of miR-1297 mimic. FA2H, fatty acid 2-hydroxylase; miR, microRNA; WT, wild-type; MUT, mutant; 3'UTR, 3'-untranslated region. **P<0.01 vs. mimic control group.

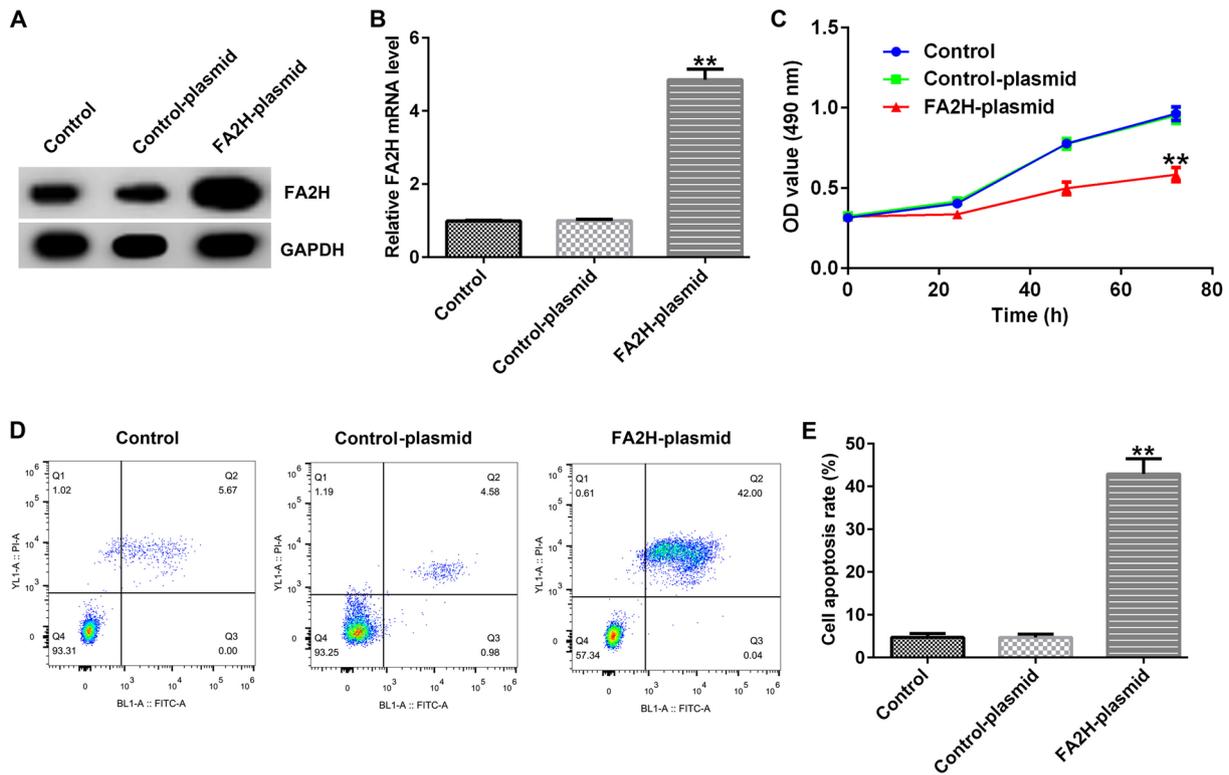


Figure 4. FA2H overexpression inhibits cell proliferation and induces apoptosis. MDA-MB-231 cells were transfected with control-plasmid or FA2H-plasmid for 48 h. The transfection efficiency of FA2H-plasmid was assessed via (A) western blotting and (B) reverse transcription-quantitative PCR. (C) MDA-MB-231 cell proliferation at 24, 48 and 72 h was measured by performing an MTT assay. Cell apoptosis was (D) determined via flow cytometry and (E) quantified. FA2H, fatty acid 2-hydroxylase; OD, optical density. **P<0.01 vs. Control-plasmid group.

E-cadherin, N-cadherin and MMP9, were assessed via western blotting and RT-qPCR. FA2H overexpression upregulated E-cadherin protein and mRNA expression levels, and decreased N-cadherin and MMP9 protein and mRNA expression levels compared with the control-plasmid group (Fig. 5A-D).

miR-1297 knockdown affects MDA-MB-231 cell proliferation by upregulating FA2H. Subsequently, the effects of miR-1297

and FA2H expression on MDA-MB-231 cell proliferation, apoptosis and EMT were investigated. MDA-MB-231 cells were transfected with control-siRNA, FA2H-siRNA, inhibitor control, miR-1297 inhibitor, miR-1297 inhibitor + control-siRNA or miR-1297 inhibitor + FA2H-siRNA for 48 h. The RT-qPCR results indicated that FA2H-siRNA significantly decreased FA2H mRNA expression levels in MDA-MB-231 cells compared with the control-siRNA group

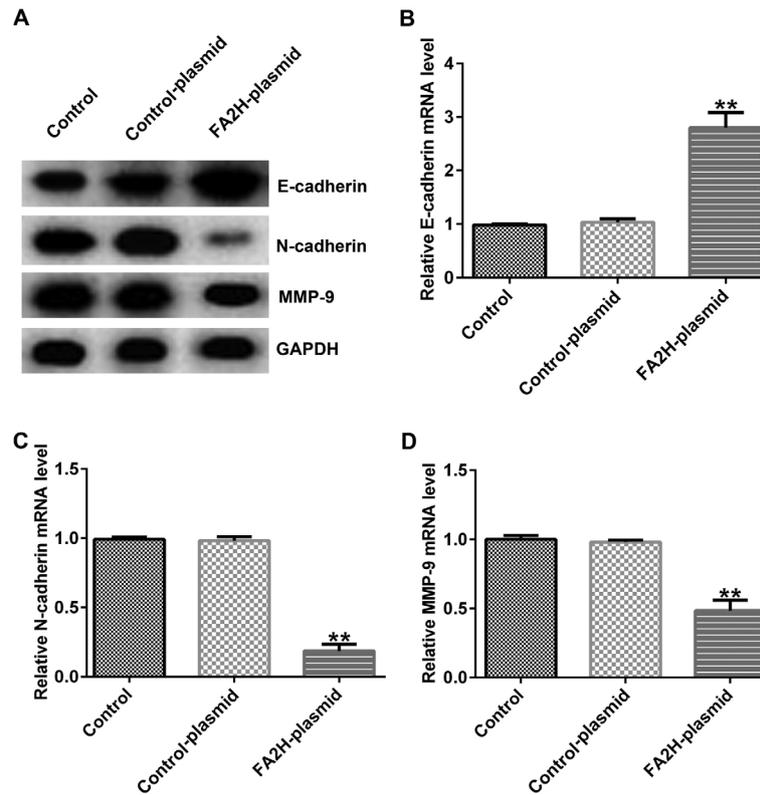


Figure 5. FA2H overexpression attenuates epithelial-mesenchymal transition. MDA-MB-231 cells were transfected with control-plasmid or FA2H-plasmid for 48 h. (A) Protein expression levels of E-cadherin, N-cadherin and MMP-9 were determined via western blotting. mRNA expression levels of (B) E-cadherin, (C) N-cadherin and (D) MMP-9 were determined via reverse transcription-quantitative PCR. FA2H, fatty acid 2-hydroxylase; MMP-9, matrix metalloproteinase-9. **P<0.01 vs. Control-plasmid group.

(Fig. 6A). In addition, compared with the inhibitor control group, miR-1297 inhibitor upregulated FA2H protein and mRNA expression levels, which were reversed by co-transfection with FA2H-siRNA (Fig. 6B and C). The MTT assay indicated that miR-1297 inhibitor significantly reduced cell proliferation at 72 h compared with the inhibitor control group (Fig. 6D). In addition, miR-1297 inhibitor significantly increased apoptosis compared with the inhibitor control group (Fig. 6E and F). miR-1297 inhibitor-mediated alterations to MDA-MB-231 cell proliferation and apoptosis were reversed by co-transfection with FA2H-siRNA.

miR-1297 knockdown affects EMT by upregulating FA2H. MDA-MB-231 cells were transfected with control-siRNA, FA2H-siRNA, inhibitor control, miR-1297 inhibitor, miR-1297 inhibitor + control-siRNA or miR-1297 inhibitor + FA2H-siRNA for 48 h. The expression levels of EMT markers were determined via RT-qPCR and western blotting. Compared with the inhibitor control group, miR-1297 inhibitor obviously increased E-cadherin protein and mRNA expression levels, and downregulated N-cadherin and MMP9 protein and mRNA expression levels (Fig. 7A-D). Co-transfection with FA2H-siRNA reversed miR-1297 inhibitor-mediated alterations to mRNA and protein expression levels.

Discussion

Several advanced biotechnology studies have demonstrated that abnormal miRNA expression in BC is a rule rather

than an exception (31,32). It has been reported that miR-21 is expressed in human BC tissues and cells (33,34), and may monitor the early occurrence of BC (35). Furthermore, miR-21 regulates cell proliferation, G₂/M checkpoints, metastatic spread (36-38) and the expression of multiple target genes, including tropomyosin-1, programmed cell death factor 4, maspin and Bcl-2 (39). Wang *et al* (40) demonstrated that miR-21 promoted BC cell proliferation and metastasis. Emerging evidence has indicated that several miRNAs are overexpressed in BC cell lines, including the miR-221/222 cluster (41), miR-9, miR10b, miR-29a, miR-96, miR-146a, miR-181, miR-373 and miR-589 (42). In addition, a previous study suggested that miR-141 is involved in the development of BC (43). Therefore, the present study aimed to investigate the role of miR-1297 in BC.

Increasing evidence has indicated that miR-1297 is associated with multiple types of cancer. Liang *et al* (44) demonstrated that miR-1297 suppressed prostate cell proliferation and invasion via the astrocyte elevated gene-1/Wnt signaling pathway (44). By contrast, other studies revealed that miR-1297 promoted cell proliferation and affected several biological behaviors in laryngeal squamous cell carcinoma and testicular germ cell tumor cells via PTEN (19,45). The present study demonstrated that miR-1297 knockdown decreased cell proliferation and increased apoptosis in MDA-MB-231 cells compared with the inhibitor control. miRNAs exert specific functions by regulating the expression of their target genes (46-48). Subsequently, bioinformatics and *in vitro* experiments were performed to verify whether miR-1297

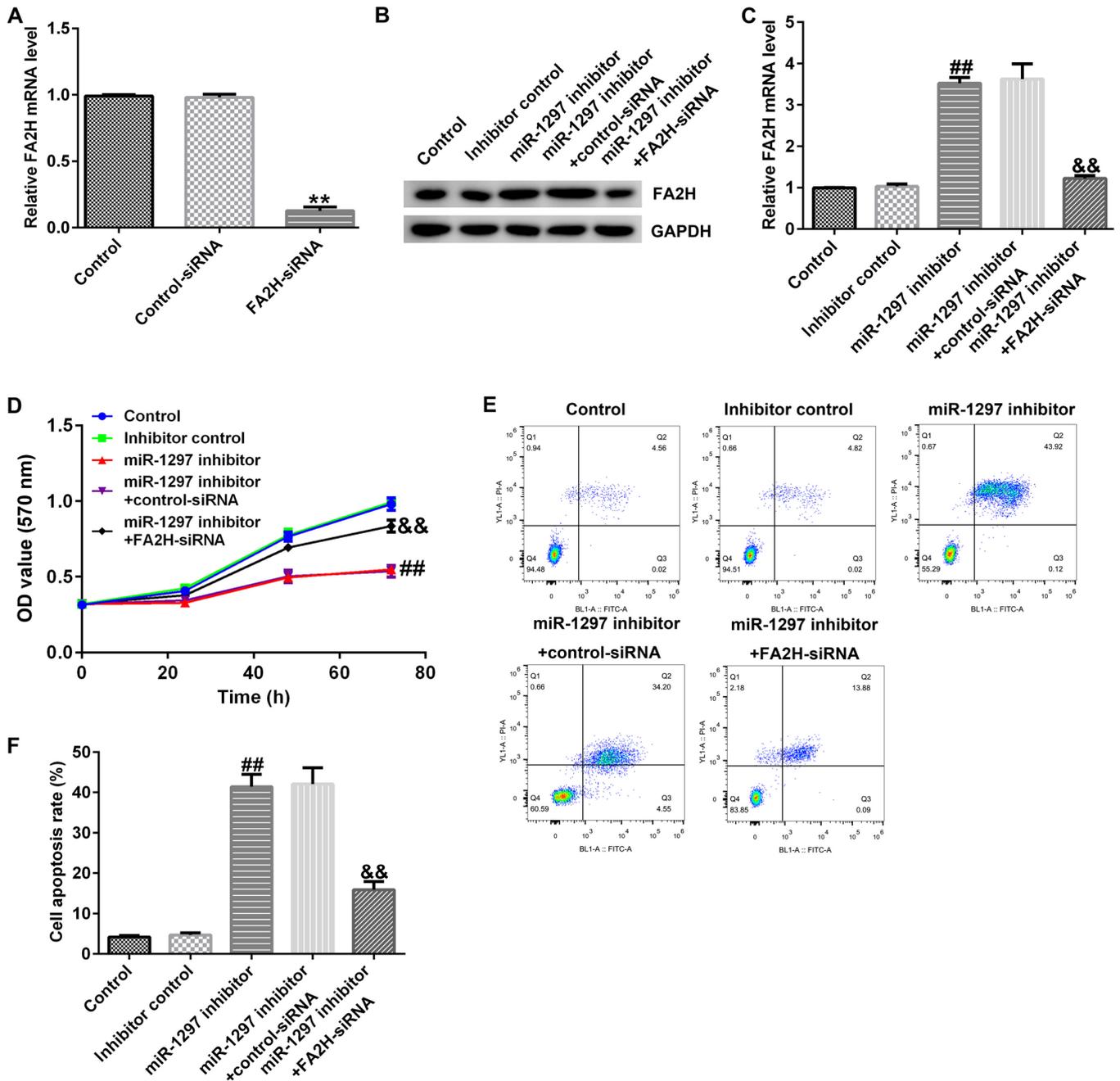


Figure 6. miR-1297 knockdown inhibits MDA-MB-231 cell proliferation and promotes apoptosis via upregulating FA2H. (A) Transfection efficiency of FA2H-siRNA. FA2H (B) protein and (C) mRNA expression levels in MDA-MB-231 cells transfected with inhibitor control, miR-1297 inhibitor, miR-1297 inhibitor + control-siRNA or miR-1297 inhibitor + FA2H-siRNA for 48 h. (D) MDA-MB-231 cell proliferation at 24, 48 and 72 h was measured by performing an MTT assay. Cell apoptosis was (E) determined via flow cytometry and (F) quantified. miR, microRNA; FA2H, fatty acid 2-hydroxylase; siRNA, small interfering RNA; OD, optical density. **P<0.01 vs. Control-siRNA group; ##P<0.01 vs. inhibitor control group; &&P<0.01 vs. miR-1297 inhibitor + control-siRNA group.

directly targeted FA2H. The results indicated that FA2H was a direct target of miR-1297.

FA2H mediates the introduction of a chiral (R)-hydroxyl group at the second carbon of long-chain FAs (24). FA2H is upregulated in multiple organs, affects cell differentiation and regulates the membrane transport capacity of nutrient transporters (49,50). Alderson and Hama (51) indicated that FA2H knockdown promoted D6P2T nerve sheath cell proliferation and suppressed cAMP-induced cell cycle arrest, suggesting that FA2H exhibited several functions in regulating signaling

pathways associated with cell proliferation. A previous study also suggested that FA2H is associated with BC (27). In the present study, FA2H overexpression decreased cell proliferation and increased apoptosis compared with the control-plasmid group. Furthermore, the results indicated that miR-1297 modulated EMT by regulating FA2H expression.

EMT is the process whereby epithelial cells are transformed into mesenchymal cells, thus gaining the ability to migrate and invade (52). EMT is an important component of cancer metastasis (53). During the early stages of cancer metastasis,

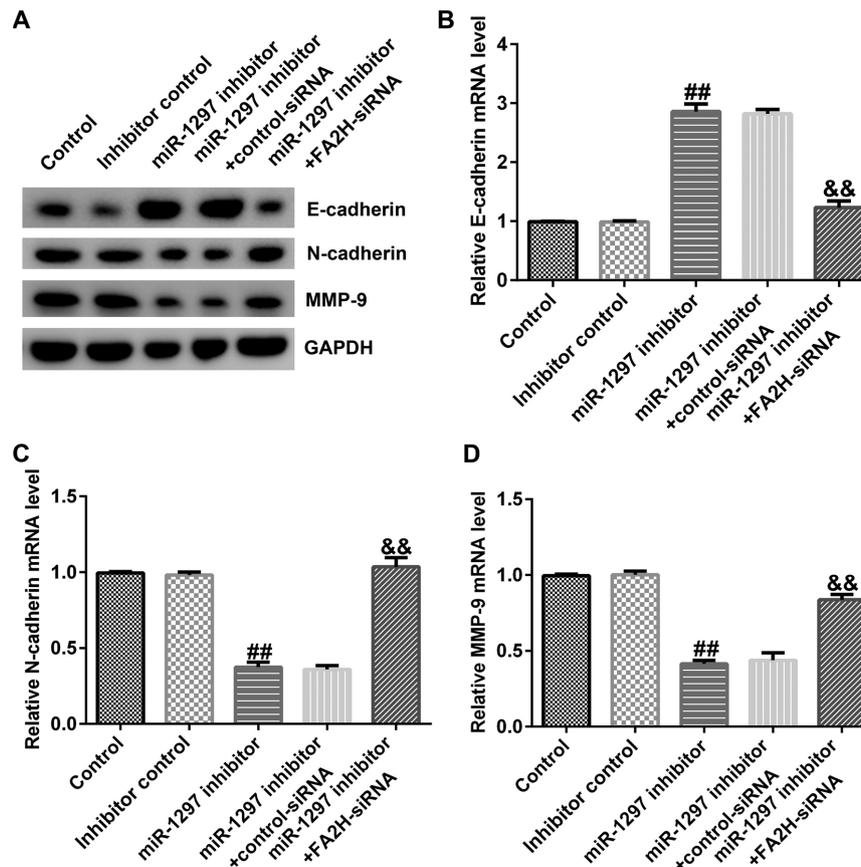


Figure 7. miR-1297 knockdown attenuates epithelial-mesenchymal transition by upregulating FA2H expression. MDA-MB-231 cells were transfected with inhibitor control, miR-1297 inhibitor, miR-1297 inhibitor + control-siRNA or miR-1297 inhibitor + FA2H-siRNA for 48 h. (A) Protein expression levels of E-cadherin, N-cadherin and MMP-9 were determined via western blotting. mRNA expression levels of (B) E-cadherin, (C) N-cadherin and (D) MMP-9 were determined via reverse transcription-quantitative PCR. miR, microRNA; FA2H, fatty acid 2-hydroxylase; siRNA, small interfering RNA; MMP-9, matrix metalloproteinase-9. ^{##} $P < 0.01$ vs. inhibitor control group; ^{&&} $P < 0.01$ vs. miR-1297 inhibitor + control-siRNA group.

separation of tumoral cells from the primary tumor may be mediated by attenuating EMT (54). The epithelial cell marker E-cadherin, and the mesenchymal cell markers N-cadherin and MMP9 are primary EMT markers (52,55). The results of the present study revealed that FA2H overexpression increased the expression levels of E-cadherin and decreased the expression levels of N-cadherin and MMP9 compared with the control-plasmid group.

Collectively, the present study indicated that miR-1297 regulated BC cell proliferation and EMT via regulating FA2H expression. Compared with the inhibitor control, miR-1297 knockdown decreased BC cell EMT and proliferation in a FA2H-dependent manner. Therefore, the present study suggested that miR-1297 and FA2H may serve as potential therapeutic targets for breast cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HL contributed to the conception and design of the study, data acquisition, analysis and interpretation, and drafted and critically revised the manuscript. BL contributed to the conception and design of the study, data acquisition, analysis and interpretation and critically revised the manuscript. JL contributed to conception and design of the study, data analysis and interpretation, and drafted and critically revised the manuscript. YL and DC contributed to data analysis and validation. All authors gave final approval and agree to be accountable for all aspects of the work. All authors read and reviewed the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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