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Research Article

MiRNA-145-5p Restrains Malignant Behaviors of Breast Cancer Cells Via Downregulating *H2AFX* Expression

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Background: Breast cancer is a prevalent tumor with high aggressiveness among female populations. *MiRNA-145-5p* plays an important role in multiple cancers.

Materials and Methods: qRT-PCR detected *miRNA-145-5p* and histone protein family member X (*H2AFX*) mRNA expression in breast cancer cells, and western blot determined the protein expression of H2AFX. After predicting the target genes via the bioinformatics methods, the targeting relationship between *miRNA-145-5p* and *H2AFX* was verified by dual-luciferase, RIP, and RNA pull-down assays. The relationship between *H2AFX* and clinical indexes was also analyzed. Furthermore, the effects of *miRNA-145-5p/H2AFX* regulatory axis on breast cancer cell progression were determined by colony formation, wound healing, CCK-8, and Transwell assays.

Results: The results suggested that miRNA-145-5p was markedly lowly-expressed in breast cancer tissue and cells, while *H2AFX* was upregulated, which had a positive correlation with T stages of breast cancer. Besides, overexpressed *miRNA-145-5p* was found to remarkably suppress progression of breast cancer cells. As bioinformatic analysis predicted that *H2AFX* was the potential target of *miRNA-145-5p*, the dual-luciferase assay was conducted, which demonstrated that *miRNA-145-5p* negatively regulated the expression of *H2AFX* by targeting its 3'-UTR. The rescue experiment demonstrated that overexpression of miRNA-145-5p could offset the promotion effects of oe-H2AFX on malignant progression.

Objective: Our study is aimed at exploring how *miRNA-145-5p* functions in breast cancer cells.

Conclusion: Our findings confirmed that *miRNA-145-5p* hindered malignant progression of breast cancer by negatively regulating *H2AFX*. *MiRNA-145-5p/H2AFX* axis may be a novel therapeutic target for breast cancer.

Keywords: Breast cancer; *H2AFX*; Invasion; *miRNA-145-5p*; Migration; Proliferation

1. Background

Breast cancer is a prevalent aggressive tumor among females. An estimated 40 thousand patients die from breast cancer annually in China, with the mortality second to only uterine cancer (1). Metastasis often occurs in many sites of breast cancer patients, mostly in the lung, brain, and bone (2-5). The improvement of early detection and treatment methods has slightly reduced the mortality of breast cancer but it still accounts for 14% of all cancer-related deaths among women (6). Hence, the corresponding mechanisms should be unveiled as a solid foundation for therapeutic target exploration.

MicroRNA (miRNA) can post-transcriptionally regulate gene expression and participate in many signaling pathways (7). Dysregulation of miRNAs has been described as

a signature in many cancers (including breast cancer) (8, 9). This study focused on *miRNA-145-5p*, a confirmed potential biomarker for risk assessment of breast cancer patients (10-12). Other studies have discovered that miRNA-145-5p acts as an anti-cancer factor in cancers. For instance, lowly expressed miRNA-145-5p in prostate cancer is suggested to promote metastasis and hence can act as the biomarker in assessing metastasis risk (13). In addition, knock-down of miRNA-145-5p promotes cancer cell proliferative and invasive abilities, which accelerates the progression of gastric cancer (14). In hepatocellular carcinoma, miRNA-145-5p reduces proliferation and migration of cancer cells while promoting apoptosis via targeting KLF5 (15). Nevertheless, since few studies shed light on the regulatory mechanism of miRNA-145-5p in breast cancer, this paper attempted to conduct an in-depth exploration to offer more theoretical reference for clinical practice.

H2A histone constitutes an octamer of core histones along with H2B, H3, and H4, surrounded by DNA (145-147 base pairs). As a variant of histone H2A, histone protein family member X (H2AFX) accounts for 2%-25% of all H2A with a varying ratio in different tissue and cells (16). Phosphorylated molecule γ-H2AFX is a biomarker of double-strand break (DSB)(17, 18), a synonym for unstable cell genome and hence regarded as a prognostic biomarker of breast cancer and other malignant tumors (17, 18). H2AFX variants are found associated with an increased risk of breast cancer(19). Therefore, profiling the specific function of H2AFX in breast cancer cells may take an essential part in improving its treatment.

This study aimed to investigate the effects of miRNA-145-5p and its downstream regulatory genes on cell proliferation, migration, and invasion in breast cancer. With *H2AFX* being predicted to be the downstream target of miRNA-145-5p via bioinformatics analysis, experimental results implied that miRNA-145-5p repressed the breast cancer cell progression by negative-ly regulating *H2AFX* expression.

2. Objective.

Our study is aimed at investigating that how miRNA-145-5p can inhibit malignant progression of breast cancer.

3. Materials and Methods

3.1. Bioinformatics Analysis

Expression data of mRNAs and clinical data (normal: 113, tumor: 1,109) were downloaded from The Cancer Genome Atlas-Breast Cancer (TCGA-BRCA) and Gene Expression Omnibus (GEO) dataset on February 10, 2021, and September 14, 2022, respectively. The samples were preprocessed before analyzing. They were merged into an expression matrix. With gtf and mature.fa annotation files retrieved from GENECODE (https://www.gencodegenes.org/) and miRBase (http://www.mirbase.org/) databases respectively, the matrices ENSG and MIMAT were annotated into genes and mature miRNAs. After extracting the mRNA expression matrix across gene types, the average expression for the same gene was calculated and miRNAs with low expression were filtered out. Differential expression analysis ($|logFC|>1.5$, $padj<0.05$) of mRNAs was performed by using the "edgeR" package. Downstream genes that had binding sites on the target miRNA were predicted by starBase, miRDB, mirDIP, and TargetScan databases, and overlapped with differentially-expressed mRNAs (DEmRNAs). Downstream target mRNA was finally ascertained by correlation analysis. Clinical staging of the target mRNA was analyzed. The mechanism of upstream miRNA and its target mRNA affecting breast cancer was investigated.

3.2. Cell Culture

Normal (MCF-10A) and breast cancer (MCF-7, T47D, MDA-MB-231 and MDA-MB-453) cell lines were cultivated in 5% CO_2 at 37 °C. The media contained 10% fetal bovine serum (FBS) (Gibco, USA), 100 U. mL^{-1} penicillin sodium (Invitrogen, USA), and 100 mg. mL^{-1} streptomycin sulphate (Invitrogen, USA). The specific information of cell lines and media is displayed in **Table 1**.

3.3. Cell Transfection

Lipofectamine 2000 (Invitrogen, USA) was used for transfection. pcDNA3.1-H2AFX plasmid (oe-H2AFX), blank pcDNA3.1 plasmid (oe-NC), miRNA-145-5p mimic (miR-mimic), mimic NC (miR-NC), and sh-H2AFX as well as its negative control (sh-NC) were transfected into MCF-7 cells at 37 °C for 48 h. Mimics and plasmids were provided by GenePharma Company (China).

3.4. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA was separated from cells with RNA (Takara, Japan), followed by being reversely transcribed into cDNAs using M-MLV reverse transcriptase (Takara,

Table 1. Information of cell lines and mediums

Cell line	Catalog No.	Medium	Company/Catalog No.
Human normal mammary epithelial cell line MCF-10A	ATCC®CRL-10317™	MEBM	Lonza/Clonetics Corporation
Breast cancer cell line MCF-7	ATCC®CRL-3435™	DMEM	ATCC Catalog No. 302002
Breast cancer cell line T47D	ATCC®HTB-133™	RPMI-1640	ATCC Catalog No. 302001
Breast cancer cell line MDA-MB-231	ATCC®HTB-26™	Leibovitz's L15	ATCC Catalog No. 302008
Breast cancer cell line MDA-MB-453	ATCC®HTB-131™	Leibovitz's L15	ATCC Catalog No. 302008

Abbreviations: ATCC: American Type Culture Collection; MEBM: mammary epithelial cell basal medium; DMEM: Dulbecco's Modified Eagle Medium; RPMI-1640: Roswell Park Memorial Institute-1640.

Japan). Afterward, the expression of miRNA-145-5p and H2AFX was determined by SYBR Green qPCR Mix (BioRad, USA) and calculated using the $2^{-\Delta\Delta Ct}$ method. The expression of miRNA and mRNA was normalized to *U6* and *GAPDH*, respectively. Shown in **Table 2** are primer sequences included.

3.5. Cell Counting Kit-8 (CCK-8)

Firstly, MCF-7 cells were transferred to 96-well plates (1×10^3 cells/well). CCK-8 kit (Dojindo, Japan) assessed cell viability at 0, 24, 48, 72, and 96 h. Absorbance at 450 nm was read with Elx800 Reader (Bio-Tek Instruments Inc., USA).

3.6. Colony Formation Assay

Transfected cells were resuspended in medium with 10% FBS at 37 °C and paved in 6-well plates (1×10^3 cells/well). After 2 weeks of incubation (the medium was replaced every three days.), cells were fixed with ethanol for 15 min and then dyed in 0.5% crystal violet for 15 min. Finally, photos were taken on the plates, and colonies with ≥50 cells were counted.

3.7. Wound Healing Assay

Cells were incubated in the serum-free medium for 24 h. Then, a 200 µL pipette tip slightly created a wound on the surface of the cell monolayer. Next, the medium was changed with a fresh one for 24 h of culture. At 0 h and 24 h, wound healing was observed and photographed. Wound healing percentage (%) = (width at 0 h-width at 24 h) / width at 0 h.

3.8. Trans well Invasion Assay

This assay was performed with a Trans well chamber (8 µm, 24-well insert; Corning, USA). Before cell invasion detection, the upper chamber was covered by diluted Matrigel (BD Biosciences, USA). Cell suspension of the serum-free medium was introduced to the upper

Table 2. Primer sequences

Gene	Forward (F) 5'-3'	Reverse (R) 5'-3'
miR-145-5p	CCTTGTCCTCACGGTCCAGT	AACCATGACCTCAAGAACAGTATTT
H2AFX	AATCTAGATCCCTTCCAGCAAACTCAAC	AATCTAGAAACTCCCCAATGCCTAAGGT
U6	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT
GAPDH	GAGTCAACGGATTTGGTCGT	TTGATTTTGGAGGGATCTCG

chamber (3×10^4 cells/well). Medium with 10% FBS was introduced to the lower counterpart. The whole cultivation lasted for 48 h. Lastly, cells invading the lower counterpart were fixed in methyl alcohol, dyed in crystal violet, and calculated under 6 random fields.

3.9. Western Blot

Proteins were separated from cells with radio immunoprecipitation assay buffer (Catalog Num.: P0013B; Beyotime, China). The bicinchoninic acid protein detection kit (Catalog No: BCA1-1KT; Sigma-Aldrich; USA) evaluated protein concentration. 50 µg proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and moved onto a nitrocellulose membrane (Bio-Rad, USA) which was immersed in 5% skim milk at room temperature for 2 h. Then it was incubated with primary antibody rabbit anti-H2AFX (1:5000, ab11175, Abcam, UK) and placed at room temperature for 2 h. Rabbit anti-GAPDH (1:10000, ab181602, Abcam, UK) was used as the control. After applying TBST detergent for three washes, we moved the membrane to the suitable horseradish peroxidase (HRP)-coupled secondary antibody goat anti-rabbit IgG H&L and cultivated at room temperature for 2 h (1:2000, ab205718, Abcam, UK). Immunoblots were then observed by chemiluminescent immunoassay (Thermo Fisher Scientific, USA). A gel imager (Bio-Rad Laboratories, USA) analyzed protein signals.

3.10. Dual-Luciferase Detection

Wild type (WT) and mutant type (MUT) H2AFX 3' untranslated regions (UTR) were inserted into several cloning sites of a luciferase reporter vector pMIR-REPORT (Thermo Fisher Scientific, USA). MCF-7 cells were firstly inoculated into 24-well plates (1×10^5 cells/well). Subsequently, miR-mimic or miR-NC (100 nM) were co-transfected with *H2AFX-WT* or *H2AFX-MUT* plasmid by using Lipofectamine 2000 (Thermo Fisher Scientific, USA). Lastly, luciferase activity was determined with a luciferase reporter kit (Promega, USA).

3.11. RNA Immunoprecipitation (RIP) Assay

The RIP assay was performed using the Imprint ® RNA immunoprecipitation kit (Millipore, USA). Beads coated with antibodies to Argonaute2 (Ago2; Millipore, USA) or immunoglobulin G (IgG; Millipore, USA) were mixed with cell extracts. Enrichment analysis of *miRNA-145-5p* and *H2AFX* was performed by qRT-PCR.

3.12. RNA Pull-Down

A biotin-labeled *miRNA-145-5p* probe and its negative control (bio-NC) were constructed. After incubating cell lysates with a probe and beads (Invitrogen, USA), enrichment of *H2AFX* was analyzed by qRT-PCR.

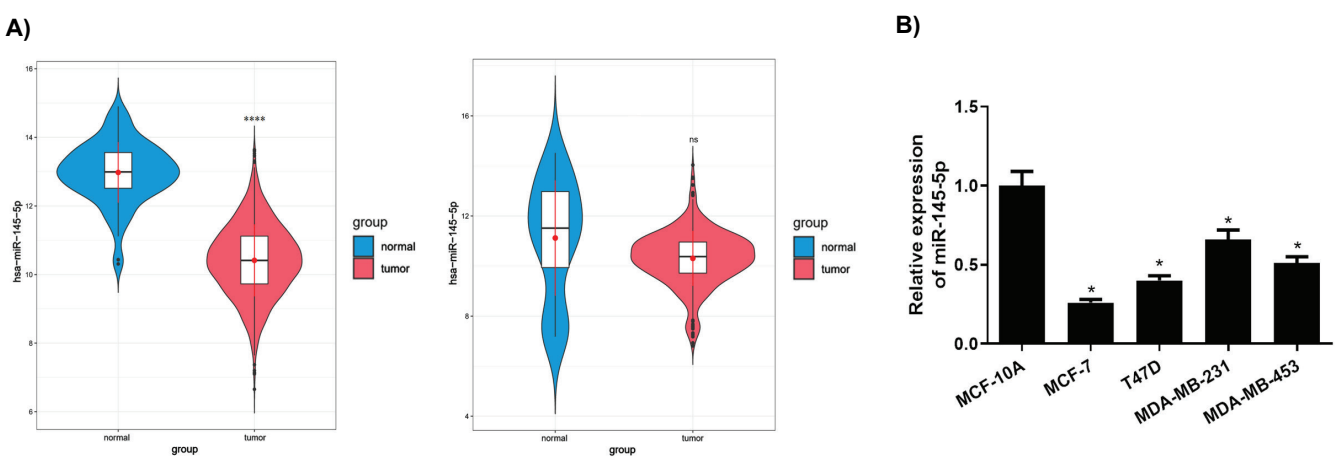

Figure 1. MiRNA-145-5p is down-regulated in breast cancer. A) Expression of miRNA-145-5p in normal tissue (blue) and tumor tissue (red) from TCGA (left) and GEO (right) databases; **B**) MiRNA-145-5p expression in normal cell line (MCF-10A) and breast cancer cell lines (MCF-7, T47D, MDA-MB-231, MDA-MB-453); * $p<0.05$, **** $p<0.0001$. ns means no significant difference.

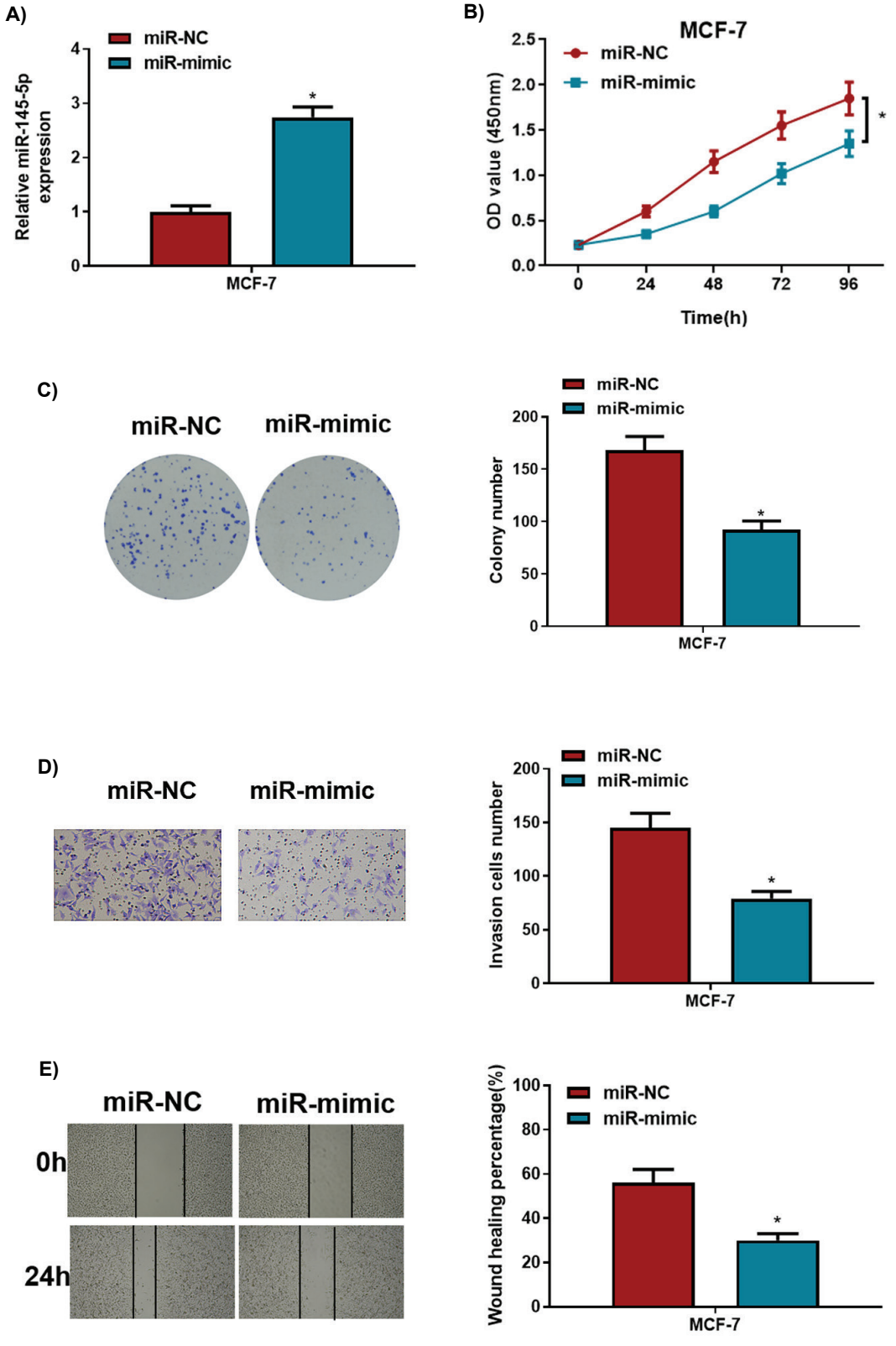

Figure 2. MiRNA-145-5p represses the progression of breast cancer cells. A) MiR-mimic and miR-NC were transfected into MCF-7 cells, and qRT-PCR evaluated the miRNA-145-5p expression in MCF-7 cells; **B**) Cell viability of MCF-7 cells; **C**) Proliferative ability of MCF-7 cells; **D**) Cell invasive ability of MCF-7 cells (100×); **E**) Cell migratory ability of MCF-7 cells (40×); * $p<0.05$.

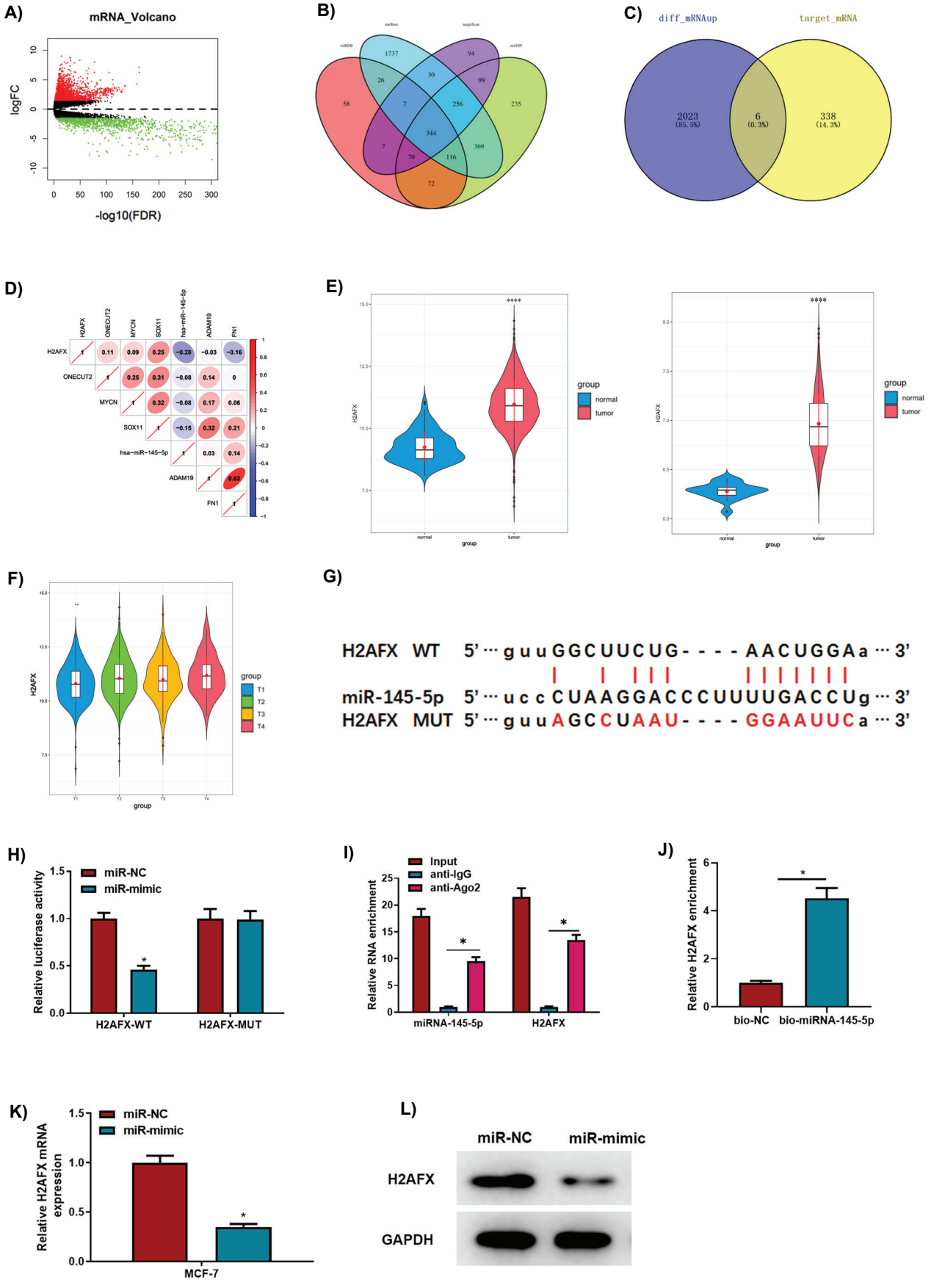

Figure 3. The legend is presented on the next page.

3.13. Data Analysis

Student's *t*-test measured the significance of the difference. Experimental data were shown as mean ± standard deviation. $p<0.05$ was considered a statistically significant difference. Data were subject to GraphPad Prism 6.0 (La Jolla, CA, USA). Each experiment underwent 3 replicates.

4. Results

4.1. MiRNA-145-5p Is Less Expressed in Breast Cancer

miRNA-145-5p was found to be less expressed in different tumor tissue and hence restrained tumor progression(20-22). Bioinformatics analysis revealed that, compared with normal tissue, tumor tissue had a remarkably down-regulated expression of miRNA-145-5p based on data from TCGA, while no significant difference was found in GEO data (**Fig. 1A**). Subsequently, a decreased expression of miRNA-145-5p was confirmed in breast cancer cells, namely MCF-7, T47D, MDA-MB-231 and MDA-MB-453 (**Fig. 1B**). Subsequent research was conducted on MCF-7 cells, which had the lowest expression of miRNA-145-5p when compared with other cell lines.

4.2. MiRNA-145-5p Suppresses the Proliferation, Invasion, and Migration of Breast Cancer Cells

By transfecting miR-NC/miR-mimic into MCF-7 cells, their transfection efficiency was measured. With its transfection efficiency detected by qRT-PCR, subsequent experiments were conducted on these transfected cells (**Fig. 2A**). CCK-8 assay showed that high miRNA-145-5p level markedly repressed cell growth (**Fig. 2B**). Colony formation assay indicated that, with the miR-NC group as the control, the colony number in miR-mimic group was prominently reduced (**Fig. 2C**). Afterward, we found that overexpressed miRNA-145-5p remarkably attenuated the invasion and migration abilities of breast cancer cells (**Fig. 2D-2E**). These observations revealed that miRNA-145-5p was a crucial suppressor of breast cancer cell proliferation, invasion, and migration.

4.3. MiRNA-145-5p Directly Targets H2AFX and Down-Regulates H2AFX Expression

Bioinformatics tools were first applied for prediction. Differential expression analysis on mRNAs using the "edgeR" package yielded 3,222 DEmRNAs (**Fig. 3A**). Target mRNAs were predicted, and 344 genes were acquired after intersection (**Fig. 3B**). Six up-regulated target mRNAs with binding sites on miRNA-145-5p were yielded (**Fig. 3C**). Pearson correlation analysis manifested that H2AFX was negatively related to miRNA-145-5p with the highest coefficient among these 6 mRNAs (**Fig. 3D**). TCGA and GEO data manifested that H2AFX was significantly highly expressed in breast cancer tissue (**Fig. 3E**). References implied that *H2AFX* is up-regulated in different tumor tissue and promotes malignant progression of tumors (23, 24). Therefore, we chose *H2AFX* for the subsequent research. Clinical information manifested that H2AFX level presented a significant difference in different clinical T stages and gradually increased as cancer progressed (**Fig. 3F**). To investigate whether *H2AFX* was a direct target of

Figure 3. *MiRNA-145-5p* directly targets *H2AFX* 3' UTR to downregulate *H2AFX* expression. A) Differential mRNAs in TCGA-BRCA. Red dots: differentially up-regulated genes, green dots: differentially down-regulated genes; **B**) Target genes of *miRNA-145-5p* predicted from databases; **C**) Venn diagram of predicted targets of *miRNA-145-5p* and up-regulated DEmRNAs; **D**) Pearson correlation analysis between *miRNA-145-5p* and 6 differential target mRNAs that had binding sites with *miRNA-145-5p*; **E**) Violin plot of *H2AFX* expression in tumor tissue (red) and normal tissue (blue) from TCGA (left) and GEO (right) databases; **F**) Violin plot of H2AFX expression in different T stages of breast cancer (blue: T1 stage; green: T2 stage; yellow: T3 stage; red: T4 stage); **G**) TargetScan predicted binding sites between *H2AFX* 3'UTR and miRNA-145-5p; **H**) Binding relationship of *H2AFX* 3'UTR and *miRNA-145-5p* (by dual-luciferase assay); **I**) RIP assay was performed to analyze the interaction between miRNA-145-5p and H2AFX. (J) RNA-pull down assay was performed to confirm the target relationship between *miRNA-145-5p* and *H2AFX*. (K-L) *H2AFX* mRNA and protein levels after transfecting miR-NC or miR-mimic; * $p<0.05$, **** $p<0.0001$.

miRNA-145-5p, we predicted potential binding sites of *miRNA-145-5p* in *H2AFX* 3' UTR through TargetScan (**Fig. 3G**). Dual-luciferase reporter further proved that *H2AFX* was the target gene of *miRNA-145-5p*. It was observed that overexpressed miRNA-145-5p markedly abated the luciferase activity of H2AFX 3'UTR WT, with no effect on that of H2AFX 3'UTR MUT (**Fig. 3H**). RIP results showed that both miRNA-145-5p and H2AFX were enriched in the precipitated complexes when the Ago2 antibody was used (**Fig. 3I**). RNA pull-down analysis illustrated that H2AFX was pulled down when the bio-miRNA-145-5p probe was used, further demonstrating the molecular interaction between *miRNA-145-5p* and *H2AFX* (**Fig. 3J**). Next, western blot and qRT-PCR determined the regulatory effects of miRNA-145-5p on H2AFX expression. We transfected miRNA-145-5p mimic or miR-NC to MCF-7 cells, implying that H2AFX mRNA and protein levels in MCF-7 cells were markedly downregulated in the miRNA-145-5p mimic group (**Fig. 3K-3L**). Together, our findings revealed that miRNA-145-5p directly targeted *H2AFX* and attenuated the expression of H2AFX in breast cancer.

4.4. H2AFX Promotes the Progression of Breast Cancer Cells

To explore the potential biological function of *H2AFX*, we transfected sh-NC or sh-H2AFX into MCF-7 cells. The transfection efficiency was measured by qRT-PCR and western blot assays. As results implied, H2AFX mRNA and protein expression was prominently inhibited in the sh-H2AFX group (**Fig. 4A-4B**).

Subsequently, we assessed the effects of H2AFX on breast cancer progression by wound healing assay, Trans well, CCK-8, and colony formation assay. As shown in **Fig. 4C-4F**, knockdown *H2AFX* hindered the progression of MCF-7 cells. To conclude, H2AFX facilitated the malignant development of breast cancer.

4.5. MiRNA-145-5p Represses Breast Cancer Cell Progression Via Regulating H2AFX

To explore the biological functions of the miRNA-145-5p/H2AFX axis in breast cancer, we performed the rescue assay. Firstly, miRNA-145-5p mimic or oe-H2AFX was transfected into MCF-7 cells, and then the transfection efficiency was detected by western blot and qRT-PCR, which suggested that H2AFX mRNA and protein expression was substantially increased in the miR-NC+oe-H2AFX group, while the expression was restored in the miR-mimic+oe-H2AFX group (**Fig. 5A-5B**). Besides, overexpression of H2AFX markedly facilitated the progressive levels of MCF-7 cells (**Fig. 5C-5F**). Though, such facilitation was impeded by the co-expression of miR-145-5p and H2AFX. Altogether, miRNA-145-5p restrained the progression of breast cancer cells by lowering the H2AFX level.

5. Discussion

Great efforts have been poured into diagnostic technology and treatment methods improvement for breast cancer. Nevertheless, the poor understanding of the molecular pathogenesis of breast cancer has been a major obstacle to researching novel treatment methods (25, 26). MiRNA-145-5p has been testified as abnormally expressed and exerts a tumor-suppressive role in malignant tumors. For instance, miRNA-145-5p hindered epithelial cell progression in gastric cancer through ANGPT2/NOD_LIKE_RECEPTOR axis (27). MiRNA-145-5p suppresses tumor cell migration, invasion, and EMT in head and neck squamous cell carcinoma via adjusting the Sp1/NF-κB signaling pathway (28). Similarly, we observed a downregulated miRNA-145-5p in breast cancer cell lines. Overexpressing miRNA-145-5p inhibited cancer cell progression, which overlapped with the results of Tang *et al*. (10) and Wang *et al.* (29). Further, existing mechanism studies implied that miRNA-145-5p/SOX2 axis, circZNF609/miRNA-145-5p/p70S6K1 axis, and LINC00052/miRNA-145-5p axis (30) are involved in mediating breast cancer progression. Differently, we collected bioinformatics data and finalized H2AFX, and revealed the mechanism of miRNA-145-5p/H2AFX axis in breast cancer for the first time.

H2AFX is expressed differently in most human cancers (31). Herein, *H2AFX* was the most potential target of miRNA-145-5p according to starBase, miRDB, mirDIP, and TargetScan databases. Previously, *H2AFX* was identified to be abnormally highly expressed in various cancers like non-Hodgkin lymphoma (32), lung cancer (33), and cervical squamous cancer (34), and was considered as a potential cancer biomarker. The regulation of miRNAs on *H2AFX* was confirmed in many studies. For example, miRNA-138 directly targets *H2AFX* to modulate DNA damage response and to repress cell proliferation in non-small cell lung cancer (24).

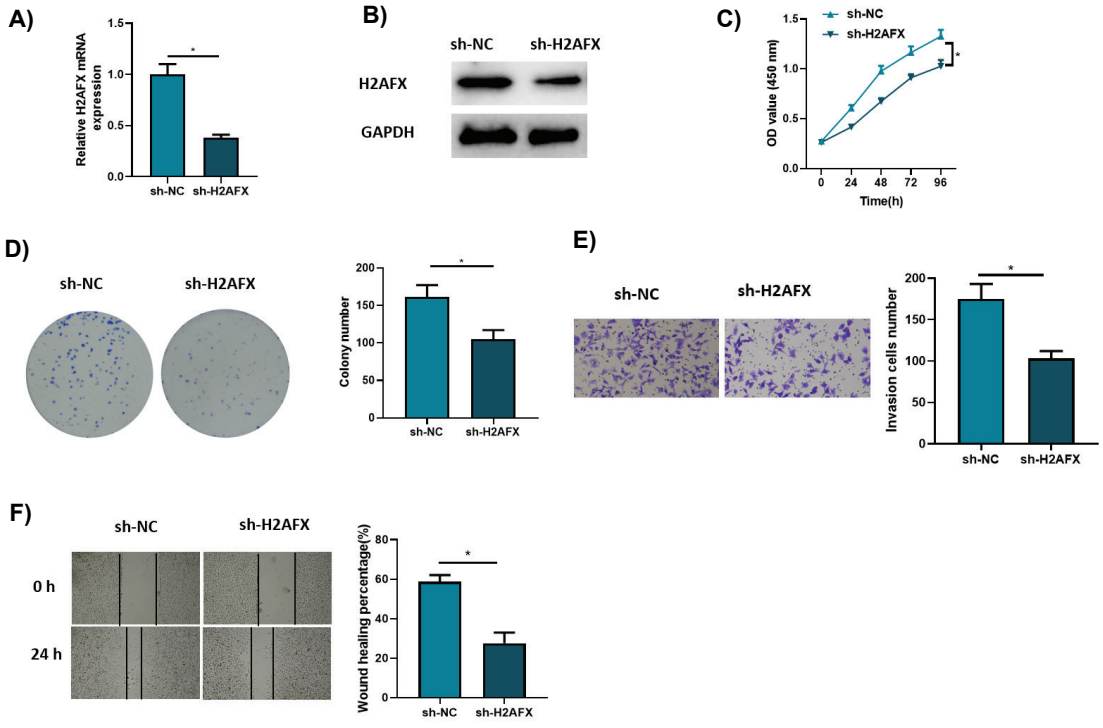

Figure 4. H2AFX facilitates the proliferation, invasion and migration of breast cancer cells. A-B) After MCF-7 cells transfected with sh-NC or sh-H2AFX, the H2AFX mRNA and protein expression was separately measured; **C**) Viability of MCF-7 cells; **D**) Proliferation ability of MCF-7 cells; **E**) Invasive ability of MCF-7 cells (100×); **F**) Migratory ability of MCF-7 cells (40×); * p<0.05.

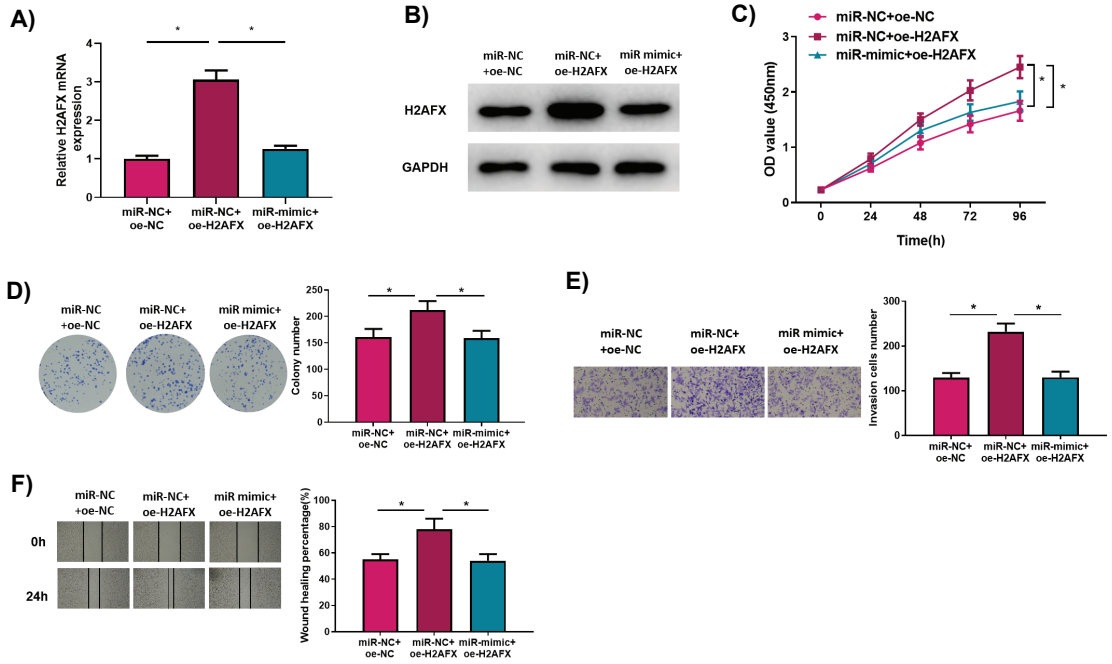

Figure 5. MiRNA-145-5p inhibits breast cancer cell progression via down-regulating H2AFX. A-B) The mRNA and protein expression of H2AFX in MCF-7 cells; **C**) Cell viability of MCF-7 cells in each treatment group; **D**) Cell proliferative ability of MCF-7 cells in each treatment group; **E**) Transwell invasion assay assessed cell invasive ability of MCF-7 cells in each treatment group (100×); **F**) Wound healing assay evaluated cell migratory ability of MCF-7 cells in each treatment group (40×); * p<0.05.

MiRNA-138 down-regulates *H2AFX* to restrain tumor growth in human cervical cancer (23). This investigation discovered that overexpressing miRNA-145-5p induced *H2AFX* down-regulation, thereby suppressing the malignant progression of breast cancer cells. Impacts of miRNA-145-5p on cell growth may partly be mediated by *H2AFX* down-regulation in breast cancer cells.

All in all, bioinformatics prediction and cellular experiments demonstrated that miRNA-145-5p re-pressed the malignant progression of breast cancer by targeting *H2AFX*. More importantly, miRNA-145-5p/H2AFX axis was strongly associated with tumorigenesis. This study also showed that revealing cancer-related miRNA biomarkers and miRNA/mRNA pathways would be the most important step for explaining breast cancer origin, and further diagnosis and treatment. Some limitations, though. To take an example, we did not validate the impact of miRNA-145-5p/H2AFX regulatory axis on the malignant progression of breast cancer *in vivo*, and we also lacked in-depth exploration of downstream pathways. In the future, we will dive deeper into the mechanism of miRNA-145-5p/H2AFX regulatory axis on breast cancer development through mice models, hoping to be part of the theoretical efforts in optimizing breast cancer treatment.

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