

Identification by Comprehensive Bioinformatics Analysis of KIF15 as a Candidate Risk Gene for Triple-Negative Breast Cancer

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Background: Previous studies have shown that kinesin family proteins (*KIFs*) play an indispensable roles in several types of cancer. However, the expression and clinical significance of *KIFs* in triple-negative breast cancer remain unclear.

Methods: In this study, the role of *KIF15*, including gene expression analysis, methylation characteristic, CNV characteristic, and miRNA target regulation, was evaluated using multiple bioinformatic tools based on TCGA database. Quantitative real-time PCR and Western blot were used to determine the expression level of *KIF15* in triple-negative breast cancer cell lines. Then, functional experiments were employed to explore the effects of *KIF15* on tumor growth and metastasis in triple-negative breast cancer.

Results: Our data showed that *KIF15* was significantly upregulated in triple-negative breast cancer (TNBC). Functionally, downregulation of *KIF15* significantly facilitated apoptosis and G2/M phase arrest, and inhibited the migration and invasion of TNBC cells. The mechanism of action of *KIF15* was closely related to DNA replication checkpoint and cell cycle regulation in TNBC based on GSEA. In addition, bioinformatics analysis demonstrated that high expression of *KIF15* in TNBC was correlated with copy number aberration and DNA methylation levels.

Conclusion: Our findings suggest that *KIF15* is a novel oncogene in TNBC and provide us a strong evidence that it might be served as a potential clinical target and biomarker in triple-negative breast cancer.

Keywords: kinesin family proteins, bioinformatics analysis, biomarker, triple-negative breast cancer

Introduction

Triple-negative breast cancer (TNBC) refers to breast cancer with negative expression of estrogen receptor (ER) and progesterone receptor (PR) and low expression of human epidermal growth factor receptor (HER2).^{1,2} Hormone receptor-negative breast cancer is characterized by low differentiation and high histological grade, which is closely related to high recurrence rate, short overall survival, and insensitivity to endocrine therapy.³ However, the prognosis of hormone receptor-negative patients is not very poor; several reports indicated that the prognosis of some patients with medullary cancer is relatively good, suggesting that it is related to the heterogeneity of the tumors.^{4,5}

TNBC is a subtype of breast cancer that lacks clinical therapeutic approaches due to the lack of corresponding therapeutic receptors and poor therapeutic efficacy of endocrine therapy and the molecular targeting of the drugs.^{6,7} Studies have shown that TNBC

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is characterized by high proportion of young patients, high histological grade, high rate of axillary lymph node metastasis, low disease-free survival (DFS) and low overall survival (OS) compared with those in non-TNBC.⁸ TNBC poses a huge challenge for clinical treatment increasing the attention of oncologists to this disease.⁹ TNBC is the result of interaction of multiple factors and multiple targets. Because of the heterogeneity of TNBC genotype, there are substantial individual differences in its histological morphology, treatment response, and prognosis. The mechanism of TNBC occurrence is still being investigated. At present, many mechanisms of action remain unclear, endocrine and bio-targeted therapies have few options, and main clinic treatments include taxanes and platinum-based chemotherapy with relatively poor efficacy. The molecular mechanism of TNBC remains unclear; however, genetics, reproductive hormones, age and other factors are associated with occurrence and development of TNBC. Activation of oncogenes and inactivation of tumor suppressor genes can induce breast cancer. Numerous studies have shown that a variety of transcription factors play an indispensable role in tumorigenesis and development.^{10–13} Transcription factors can specifically control the expression of a large number of the downstream genes regulated by the upstream signals to eventually determine the fate of the cells.^{14,15}

Kinesin superfamily proteins (KIFs) are a class of molecular motors that hydrolyze ATP to produce energy to transport intracellular vesicles, organelles, RNA, and other substances.^{16,17} Kinesin family member 15 (*KIF15*) is a member of the kinesin-12 subfamily, also known as *HKLP2*, which is another tetrameric spindle motor in addition to kinesin-5.¹⁸ Studies^{19–21} have shown that *KIF* is related to the development of breast, lung, and pancreatic cancer. However, changes in the expression of other members of the *KIF* family in TNBC and the impact of these changes on the prognosis of patients have not been reported in detail. This study is the first to comprehensively analyze the transcriptional level and prognostic significance of *KIF15* in 35 human cancers using an online TCGA analysis database. The results of bioinformatics analysis and functional experiments suggest that *KIF15* is a novel oncogene in TNBC and that *KIF15* may be a clinical biomarker of TNBC.

Methods

Use of the GEPIA Database for Data Extraction

GEPIA (<http://gepia.cancer-pku.cn/>) contains the RNA sequencing expression data of 9736 tumor and 8587

normal samples of 33 malignant tumors from TCGA and GTEx.²² In this study, the GEPIA database was used to estimate the differences in *KIF15* expression in various tumor types by the “Correlation Analysis” module.

LinkedOmics Data Analysis

After logging in to LinkedOmics database homepage,²³ “TCGA_BRCA” was selected as cancer type, and “HiSeq_RNA” was selected to retrieve the dataset. The input gene was “*KIF15*”, and “Clinical” was selected for the target dataset. Finally, “Non-parametric *t*-test” was selected as statistical method; the query was submitted and the results of the analysis were downloaded at $P < 0.05$. LinkedOmics was used to analyze the correlation between the changes in *KIF15* mRNA levels, *KIF15* copy number, and methylation.

Gene Set Enrichment Analysis (GSEA)

GSEA software was used (<http://www.broadinstitute.org/gsea/index.jsp>). The RNA sequence data of the TCGA-TNBC cohort were analyzed and divided into the low or high *KIF15* expression groups according to the median expression of *KIF15*. The purpose of GSEA analysis was to avoid missing genes and pathways that play a key role in the screening of differentially expressed genes. The absolute value of the normalized enrichment score (NES) > 1 and the nominal p value < 0.05 were considered as the thresholds of statistical significance.

Cell Culture and Transfection

Human TNBC cell lines MDA-MB-231, MDA-MB-468, BT-549, MDA-MB-436, and HCC-1937 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). MDA-MB-231 and stable *KIF15*-knockdown MDA-MB-231 cells were cultured in DMEM high glucose medium (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum. MDA-MB-436 and MDA-MB-468 cells were cultured in L-15 medium containing 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, USA). BT-549 and HCC-1937 cells were cultured in RPMI 1640 medium (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum. These five cell lines were incubated in a humidified incubator with 5% CO₂ at 37°C. The culture medium was replaced every 2 days, and cells in logarithmic growth phase were selected for the experiments. At 90% confluence, the cells were passaged (1:3) or used in the experiments.

The cells were seeded into 6-well plates (2×10^3 cells/well); after 24 h of culture, the cells were supplemented with pLOV-CMV-shRNA (interference with *KIF15* expression) and pLOV-CMV-GFP (negative control) lentiviruses, at a multiplicity of infection (MOI) of 30 in combination with 5.0 $\mu\text{g}/\text{mL}$ polybrene (Gibco). After 24 h, the medium was changed, and the cells were passaged in the presence of puromycin (Gibco) to establish stable infected cells. The control shRNA sequence was 5'-TCCACGTAACCTCCGAGAAT-3' and the *KIF15*-specific shRNA sequence was 5'-TCCAGCATGAATCCCGAAT-3'.

Cell Cycle Assay by Flow Cytometry

Cells in logarithmic growth phase (10^4) were inoculated into 24-well plates and cultured for specific time for the experiments. The cells were centrifuged at 800 rpm for 5 min, and cell precipitate was collected. Then, the supernatant was discarded and precooled PBS was used to wash the cells twice. Precooled 75% ethanol was added, and the cell precipitate was fixed at 4°C for at least 4 h. Cells were centrifuged at 1500 rpm for 5 min; the supernatant was discarded, and the pellet was washed with 3 mL PBS once; 400 μL PI solution and 100 μL RNase A (100 $\mu\text{g}/\text{mL}$) were added, and the samples were incubated at 4°C in the dark for 30 min. The cells (20,000 to 30,000) were detected by standard methods, and the results were analyzed by the ModFit software. All experiments were performed three times.

Apoptosis Assay by Flow Cytometry

MDA-MB-231 cells were seeded into 6-well plates (2×10^5 cells/well) and cultured for 24 h. After digestion with trypsin, the cells were placed in a centrifuge tube and centrifuged at 2000 rpm for 5 min, and the supernatant was removed. Phosphate buffered saline (PBS) was used to wash the cells 3 times. Cells were resuspended in 400 μL binding buffer. Annexin V-FITC (5 μL) was added, and the samples were incubated at 4°C in the dark for 20 min. Apoptosis of the cells was assessed by flow cytometry after the addition of 5 μL of PI for 5 min before the assay. The experiments were repeated three times.

Scratch Wound Assay

MDA-MB-231 cells were seeded in 6-well plates (2×10^5 cells/well) and cultured for 24 h. The back side of the plate was marked with a marker pen and a ruler was used to draw even horizontal lines approximately every 0.5–1 cm across the well. The cells (5×10^5) were seeded into the

wells. On the next day, a straight line was drawn with a pipette tip perpendicular to the line on the bottom. The cells were washed with PBS 3 times to remove the detached cells, and serum-free medium was added. The dishes were placed in a 37°C incubator with 5% CO_2 and imaged at 0, 6, 24, and 48 h. All experiments were repeated three times.

Transwell Assay

MDA-MB-231 cells were seeded into 6-well plates (10^5 cells/well) and cultured for 24 h. Cells were serum-starved for 12–24 h before preparation of cell suspension to remove the serum. Cell suspension (100 μL) was added to a Transwell chamber (Costar, USA). In the lower chamber of a 24-well plate, 600 μL of medium containing 20% FBS was added, and the samples were incubated for 12–48 h. The Transwell chamber was removed, and the medium inside it was discarded. The chamber was washed twice with calcium-free PBS, fixed with methanol for 30 min, and dried. After 0.1% crystal violet staining for 20 min, the upper side of the membrane containing unigrated cells was gently wiped with a cotton swab and washed with PBS 3 times. The cells on the bottom side on the membrane were then observed in five fields of view at 400-fold magnification using a microscope. All experiments were performed three times.

Western Blot

Cells were lysed in protein lysis buffer, vortexed on ice for 10 min (vortexing for 30 s and incubation on ice for 15 s) and centrifuged at 4°C at 12,000 rpm for 5 min. The supernatant (total 20 μg of protein) was mixed with loading buffer and boiled for 5 min. Subsequently, 5% stacking and 10% separation gel was prepared for electrophoresis. After electrophoresis at 120 V for 60 min and 350 mA for 60 min, the proteins were transferred on the PVDF membranes. Then, the membranes were incubated with primary anti-*KIF15* (#ab272615, Abcam, 1:1000) and anti-vinculin antibodies (#ab219649, Abcam, 1:500) and with secondary antibodies. Relative grayscale intensity of the bands was analyzed using the ImageJ software. Tubulin was used as the internal standard.

Real-Time PCR (RT-PCR)

Total cellular RNA was extracted using TRIzol reagent (Invitrogen, USA) and stored in a -80°C freezer. The extracted RNA was subjected to reverse transcription using a cDNA first-strand synthesis kit (TaKaRa, PrimeScript RT

Master Mix, RR036A). Reversed transcription was performed at 37°C for 15 min and 85°C for 5 s; the cDNA was stored at 4°C. PCR amplification of the target genes and the internal reference GAPDH was carried out using SYBR Premix Ex Taq II, RR820A (Takara, Dalian, China). Primer sequences of *KIF15* were as follows: forward, 5'-CTCTCACAGTTGAATGTCCTTG-3', and reverse: 5'-CTCCTTGTCAGCAGAATGAAG-3'. Primer sequences of *GAPDH* were as follows: forward, 5'-AGAAGGCTGGGGCTCATTTG-3', and reverse: 5'-AGGGGCCATCCACAGTCTTC-3'. RT-PCR was performed at 95°C for 30 s, 95°C for 5 s, and 60°C for 30 s, for a total of 40 cycles. Expression level of the target gene was calculated by the $2^{-\Delta\Delta Ct}$ method. All experiments were performed three times.

Statistical Analysis

The differences in *KIF15* expression in cancer and normal tissues were analyzed by EDGER; *KIF15* expression in various different clinical stages was analyzed by one-way ANOVA. The correlation study used Pearson correlation analysis; the associations between *KIF15* expression and patient prognosis were analyzed by Kaplan–Meier method. The survival in the two groups was analyzed by Kaplan–Meier method. Log rank test was used to compare the rates, and $P < 0.05$ indicated that the differences were statistically significant.

Results

KIF15 is Abnormally Expressed in Various Types of Human Pan-Cancer

In this study, the expression profiles and prognostic value of *KIF15* were comprehensively analyzed in 35 cancer types based on the online TCGA analysis database. *KIF15* was found to be abnormally expressed in 30 types of cancers, including adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma (CESC), and colon adenocarcinoma (COAD) (Figure 1A). These data indicate that *KIF15* is abnormally expressed in various types of human cancer. Specially, the expression level of *KIF15* in the triple-negative breast cancer tissues was higher than that in the normal breast tissues (116 TNBC and 290 normal breast tissues); the corresponding statistical difference is shown in Figure 1B.

Expression of KIF15 in TNBC Cells

To verify the expression of *KIF15* in TNBC, we cultured various TNBC cell lines (HCC-1937, MDA-MB-231,

MDA-MB-436, MDA-MB-468, and BT549). The expression of *KIF15* in cultured human TNBC cells was detected by real-time quantitative PCR. As shown in Figure 2A, the expression of *KIF15* at the mRNA level was significantly higher in MDA-MB-231 cells than that in other cell lines. The protein expression levels of *KIF15* in the five cell lines were detected by Western blot. The results were consistent with the expression levels of mRNA as shown in Figure 2B.

Then, MDA-MB-231 cells were selected for infection with lentiviruses in the NC, sh*KIF15*RNA-1 group, sh*KIF15*RNA-2 group, and sh*KIF15*RNA-3 groups. As shown in Figure 2C and D, the effect of knockdown in the sh*KIF15*RNA-1 group was the highest, and a series of subsequent experiments were performed in the sh*KIF15*RNA-1 group.

Effect of KIF-15 on Apoptosis of MDA-MB-231 Cells

To determine the role of *KIF15* in apoptosis of TNBC cells, Annexin V/PI double staining was used to evaluate the influence on *KIF15* on apoptosis of MDA-MB-231 cells and MDA-MB-231/sh*KIF15*RNA-1 cells. The results showed that apoptosis in MDA-MB-231/*KIF-15* knockdown cells in the experimental group was significantly augmented compared with that in the control group, and the difference was statistically significant ($P < 0.05$, Figure 2E). This result suggests that apoptosis and *KIF15* expression are significantly correlated, and silencing *KIF15* can significantly facilitate apoptosis in MDA-MB-231 cells. Additionally, infected with *KIF-15* shRNA induced the G2/M phase arrest in MDA-MB-231 cells (Figure 2F), indicating that cell cycle regulation by *KIF15* plays an important role in MDA-MB-231 cells.

KIF15-Related Upstream Mechanisms

miRNAs potentially targeting *KIF15* in TNBC were then investigated. Five independent online tools (miRMap, miRDB, TargetScan, miTarBase, and miRanda) were used to predict possible miRNAs targeting *KIF15*, and 10 candidate miRNAs were identified (Figure 3A, Supplementary Table S1). *Hsa-miR-135a-5p*, *hsa-miR-139-5p*, *hsa-miR-381-3p*, *hsa-miR-664a-5p*, *hsa-miR-2115-5p* were correlated with *KIF15* expression (Figure 3B–F).

LinkedOmics analysis showed that *KIF15* overexpression is positively correlated with its copy number aberration in TNBC ($r = 0.358$, $P < 0.01$, Figure 3G), and *KIF15*

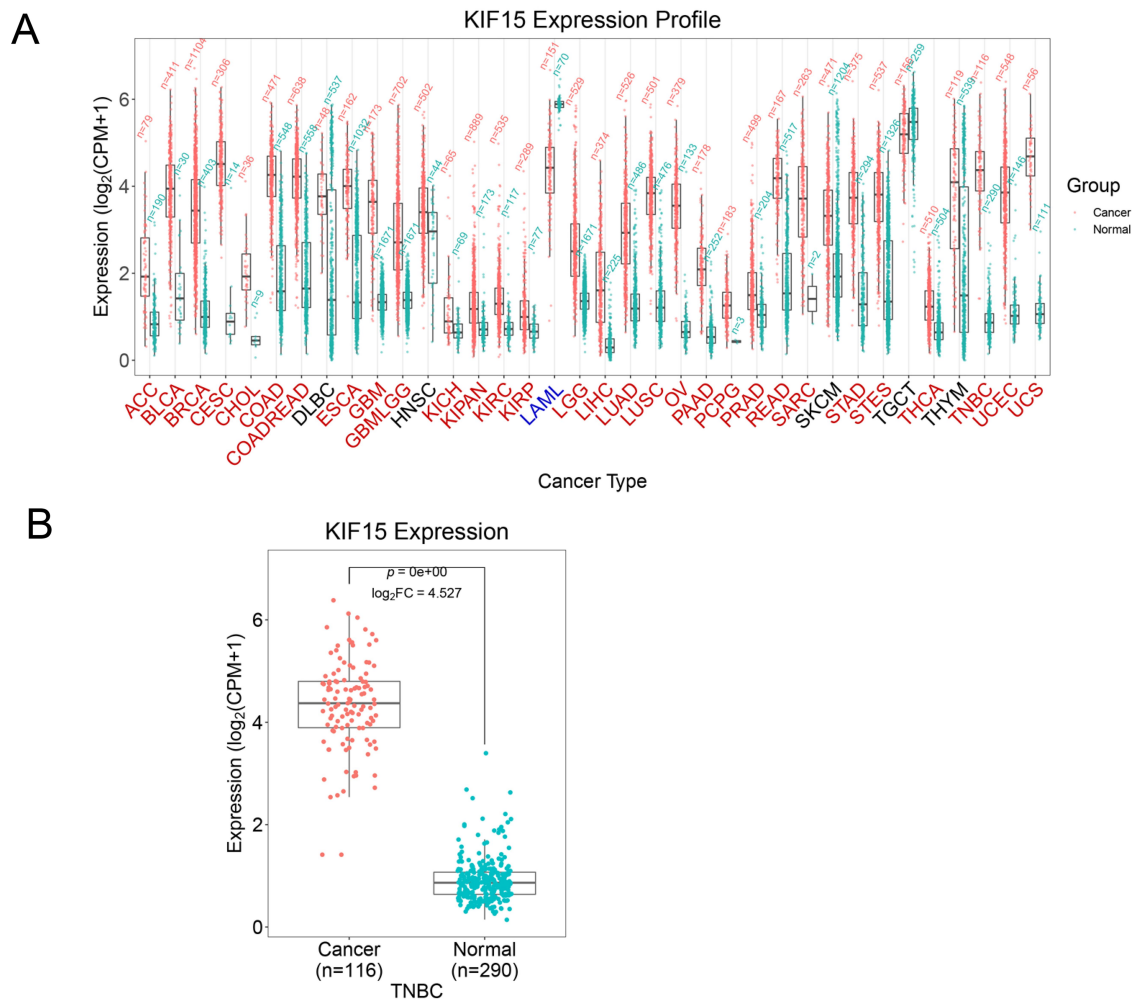


Figure 1 The mRNA level of *KIF15* in various types of human pan-cancer (GEPIA). **(A)**, *KIF15* is upregulated in 29 and downregulated in 1 type of cancer. Red and the green bar graphs refer to tumour (T) and normal tissues (N), respectively. **(B)**, The mRNA level of *KIF15* in human TNBC and normal tissues.

overexpression was negatively correlated with the level of DNA methylation of the *KIF15* gene ($R^2 = 0.239$, $P < 0.01$, Figure 3H).

Effect of *KIF15* on the Migration and Invasion Ability of MDA-MB-231 Cells

MDA-MB-231 cells infected with lentivirus were used to detect the changes in migration in the three groups at 6 h, 24 h, and 48 h after the scratching. The results showed that the distance between the scratches was significantly lower in the control group; in the *KIF15* knockdown group, the distance between the scratches was not significantly lower. There were significant differences between time periods and with the control group (Figure 4A). To explore the influence of *KIF15* on the migration and invasion of MDA-MB-231 cells, the effect of *KIF15* knockdown was assessed by the transwell invasion assay. The results

indicated that the number of cells invading the lower compartment cells was significantly reduced in MDA-MB-231/*KIF15* knockdown compared with that in the control group at 24 h and 48 h (Figure 4B). Thus, cell invasion was significantly reduced after silencing *KIF15* in MDA-MB-231 cell.

KIF15-Related Downstream Mechanisms

Investigation of the molecular mechanism of *KIF15* in the occurrence and development of TNBC plays an indispensable role in the treatment of the disease and can guide targeted clinical interventions, improving the prognosis of the patients. Genes co-expressed with *KIF15* in breast cancer were analyzed using the LinkedOmics database, and a PPI network centered on *KIF15* was generated. The proteins associated with *KIF15* were *NCAPG*, *KIF23*, *KIF20A*, *KIF2C*, *RACGAP1*, *CENPE*, *KIF4A*,

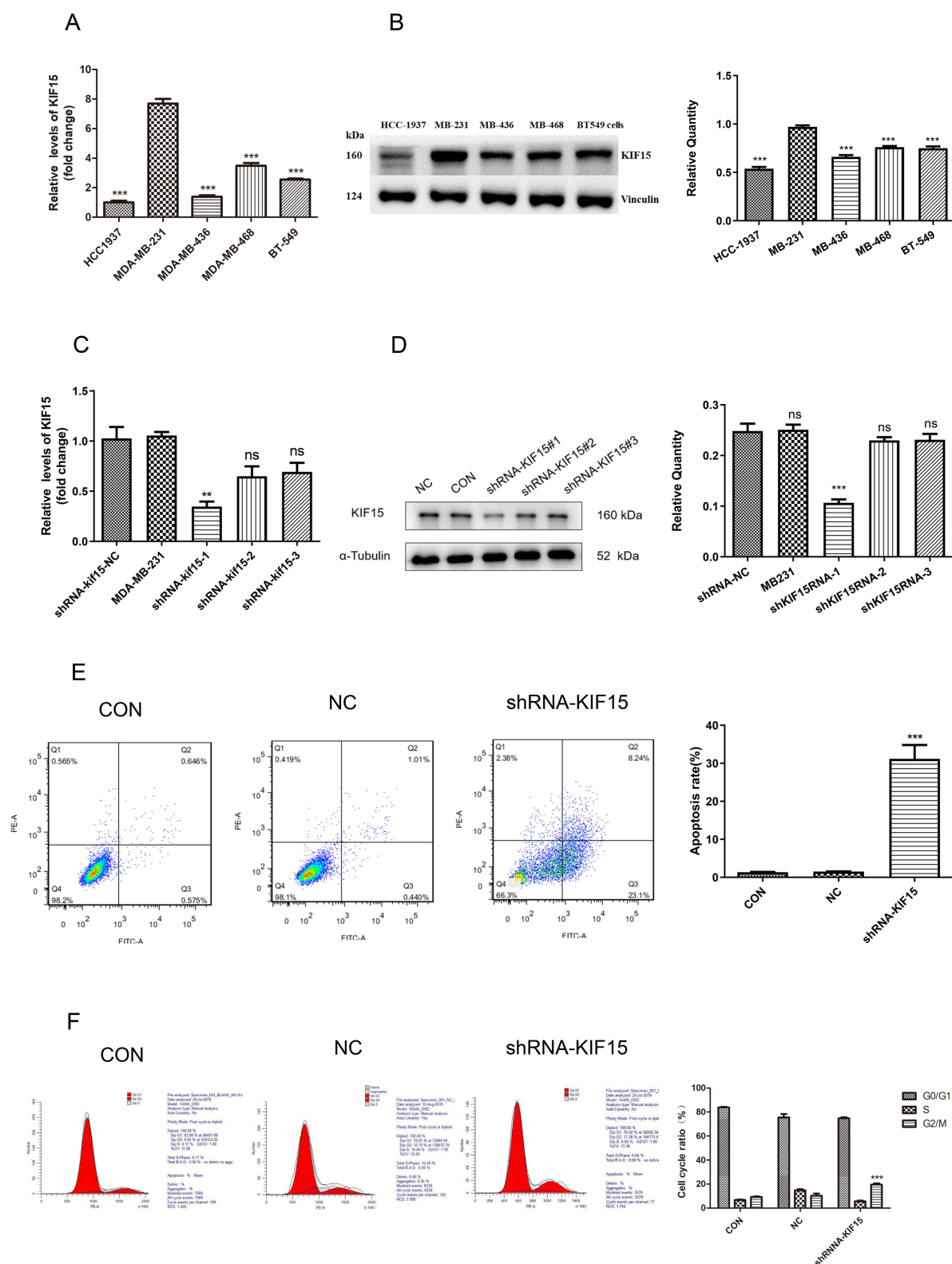


Figure 2 The effects of *KIF15* knockdown on cell cycle and apoptosis in MDA-MB-231 cells (A) Transcriptional expression of *KIF15* in various triple-negative breast cancer cell lines. (B) Western blot images (left) and quantification (right) of *KIF15* at the protein level in various triple-negative breast cancer cell lines. (C) *KIF15*-knockdown MDA-MB-231 stable cell lines, and qPCR confirming the knockdown. (D) Western blot images (left) and quantifications (right) of the efficiency test of *KIF15*-knockdown MDA-MB-231 stable cell lines. (E) Detection of apoptosis of MDA-MB-231 cells by Annexin V/PI double staining. (F) Detection of the cell cycle of MDA-MB-231 cells by PI staining. **P < 0.01. ***P < 0.001.

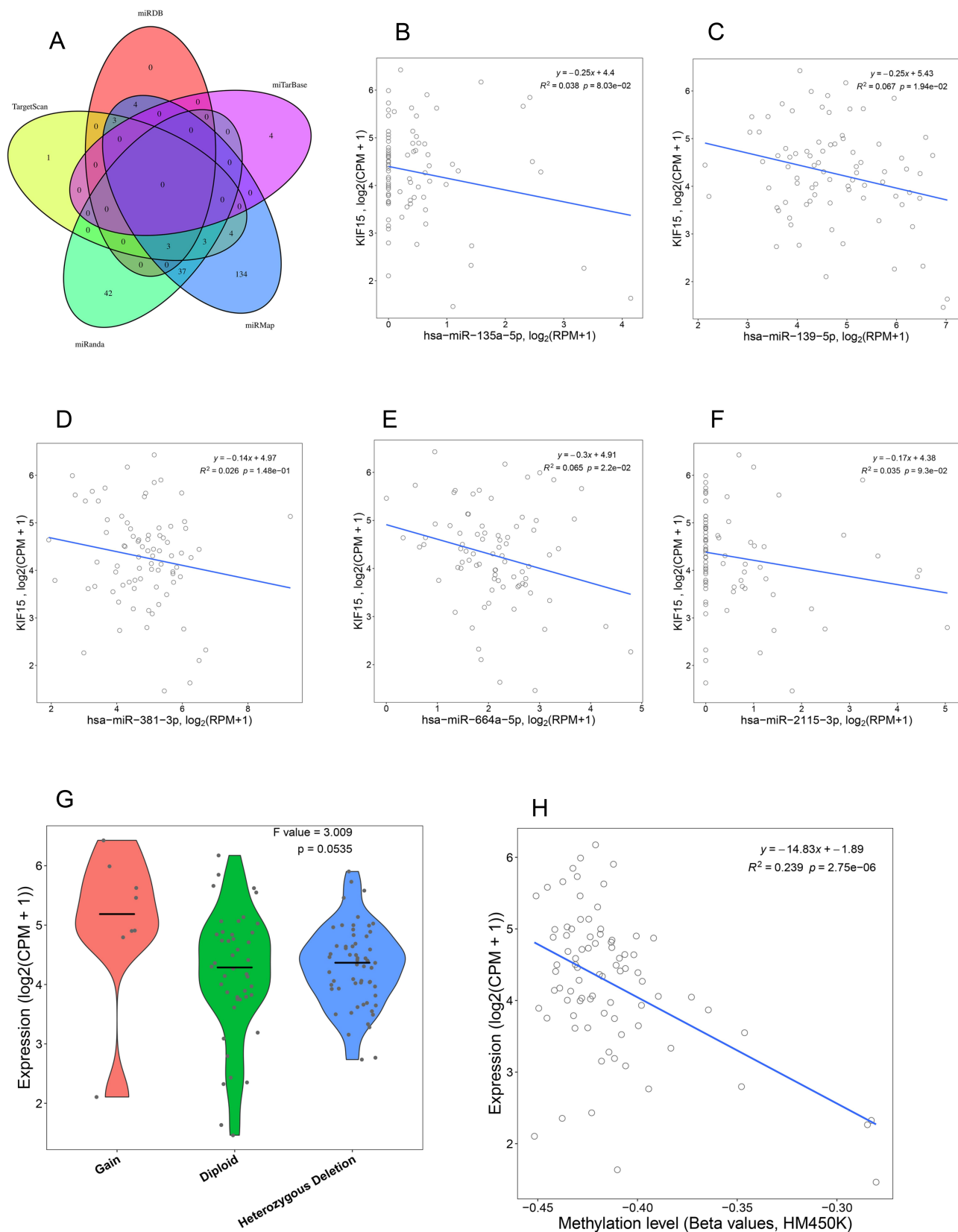


Figure 3 *KIF15*-related upstream mechanisms. **(A)** Five online tools were used to predict miRNAs targeting *KIF15*. The correlations between *KIF15* and *hsa-miR-135a-5p* **(B)**, *hsa-miR-139-5p* **(C)**, *hsa-miR-381-3p* **(D)**, *hsa-miR-664a-5p* **(E)**, *hsa-miR-2115-5p* **(F)** in breast cancer were analyzed. Copy number change **(G)** and methylation level **(H)** and of *KIF15* is associated with mRNA expression.

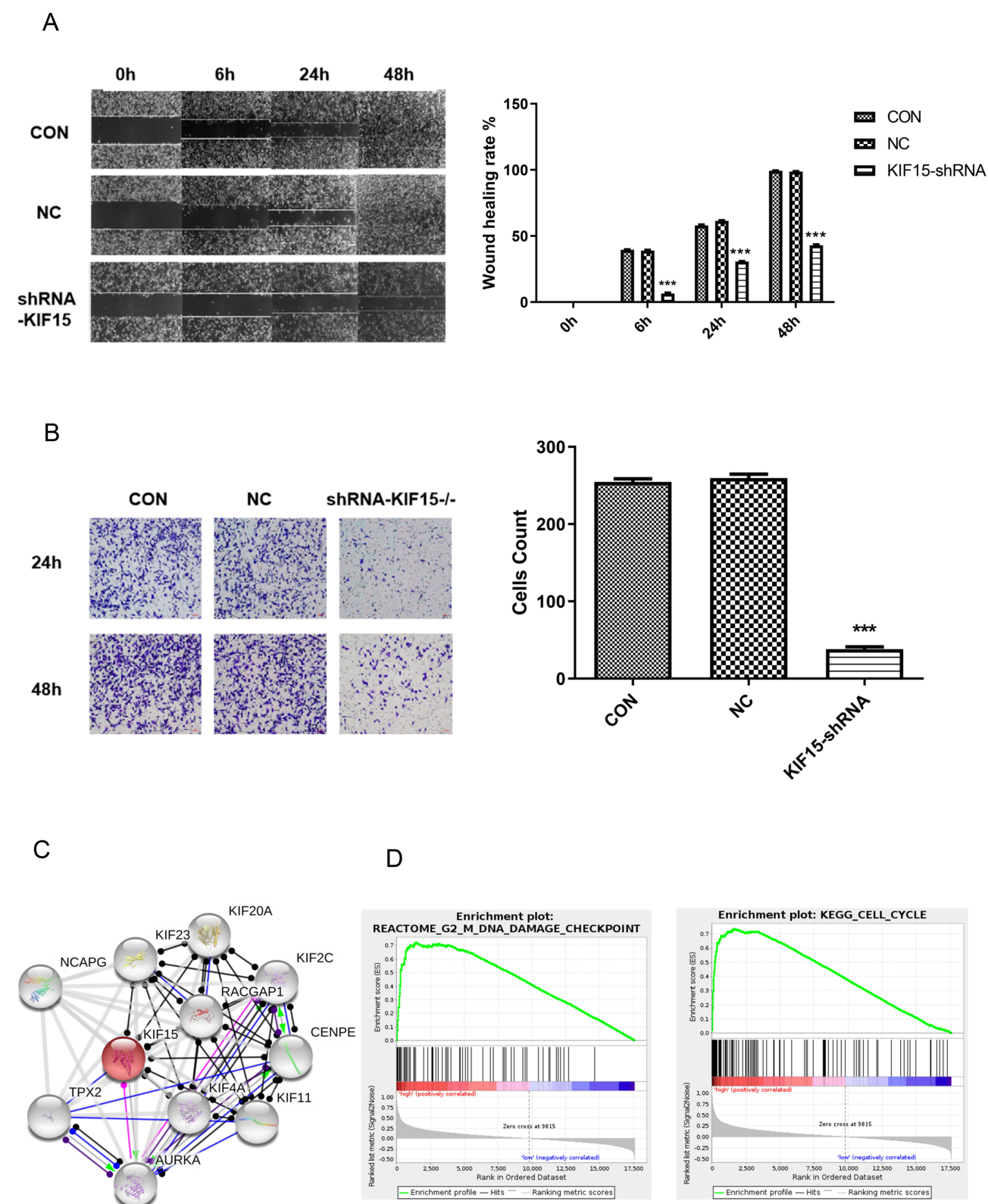


Figure 4 The effects of *KIF15* knockdown on the migration and invasion in MDA-MB-231 cells. **(A)** The effects of *KIF15* knockdown on migration (images on the left and quantification on the right) in MDA-MB-231 cells. **(B)** The effects of *KIF15* knockdown on invasion (images on the left and quantification on the right) in MDA-MB-231 cells. **(C)** The protein-protein interaction network in breast cancer. **(D)** Two pathways (DNA damage and cell cycle) are associated with *KIF15* in breast cancer according to GSEA. FDR is the false discovery rate, and the NES is the normalized enrichment score. *** $P < 0.001$.

KIF11, *AURKA*, and *TPX2* (Figure 4C). DNA-binding of transcription factors according to the ChIP-seq data of the ENCODE database predicted the transcription factor relationships regulating *KIF15* (Supplementary List S1).

To investigate possible pathways mediated by *KIF15*, GSEA of the genes coexpressed in TNBC was performed. The results of GSEA showed that *KIF15* is mainly involved in DNA damage checkpoint (P=0, FDR=0, NES=2.84) and cell cycle (P=0, FDR=0, NES=1.96) in TNBC (Figure 4D). The top 20 pathways with the most obvious up-regulation and down-regulation are listed in Tables 1 and 2.

Discussion

Various factors such as genetics, age, and reproductive hormones are significantly associated with the occurrence and development of breast cancer. Overall, activation of the oncogenes and inactivation of the tumor suppressor genes can induce breast cancer. Mutations in the tumor suppressor genes, such as *BRCA1*, and activation of the oncogenes, such as *ERα*, *Her-2*, and *beta-catenin*, can facilitate breast cell proliferation, survival, and metastasis.²⁴ Transcription factors integrate the upstream signals and specifically control the expression of numerous downstream genes thus determining the fate of the cells.^{25,26}

Recently, a large number of studies^{27,28} have shown that *KIFs* are a conserved class of microtubule-dependent

molecular motor proteins with ATPase activity, which are associated with neurodegenerative diseases, metabolic diseases, nephropathy, and other diseases. Additionally, *KIFs* play an essential role in the development of tumors.²⁹ Studies have demonstrated^{30,31} that during tumor cell division, abnormally expressed *KIFs* may affect tumor cell proliferation, invasion, and metastasis by influencing chromosome agglutination and spindle formation. For example, *KIF1B* facilitates glioma cell migration and invasion by inducing membrane-type matrix metalloproteinases.³² In lung cancer cells, downregulation of *KIF23* facilitates apoptosis, and *KIF23* is considered a potential therapeutic target for lung cancer patients.³³ Chen et al³⁴ reported that some *KIF* family members are associated with TNM stage in HCC patients, and the expression of *KIF15* and *Ki-67* in tumor tissues is positively correlated. *KIF* is involved in essential biochemical processes including mitosis, suggesting that *KIF15* may affect cell proliferation by influencing mitosis.

Given that *KIF15* is upregulated in TNBC, we investigated the functions and possible mechanisms of *KIF15* in breast cancer. GSEA indicated that *KIF15* may be involved in DNA replication and cell cycle in TNBC. It is known that DNA replication is an essential biological event in cell proliferation. The in vitro data indicate that *KIF15* is involved in the proliferation of TNBC cells. Considering that *KIF15* may facilitate the development

Table 1 The GSEA Analysis Results Showed That the First 20 Pathways Were Significantly Enriched

NAME	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_CELL_CYCLE_MITOTIC	0.67000663	2.4948568	0	0	0
REACTOME_M_PHASE	0.65098697	2.471997	0	0	0
REACTOME_RESOLUTION_OF_SISTER_CHROMATID_COHESION	0.75624627	2.4580603	0	0	0
REACTOME_MITOTIC_PROMETAPHASE	0.7204998	2.4528244	0	0	0
REACTOME_RHO_GTPASES_ACTIVATE_FORMINS	0.6845926	2.442813	0	0	0
REACTOME_NUCLEAR_ENVELOPE_BREAKDOWN	0.74754137	2.432313	0	0	0
PID_ATR_PATHWAY	0.8373444	2.420598	0	0	0
REACTOME_CELL_CYCLE_CHECKPOINTS	0.69488734	2.406858	0	0	0
REACTOME_TRANSPORT_OF_MATURE_TRANSCRIPT_TO_CYTOPLASM	0.7458618	2.4044647	0	0	0
REACTOME_MITOTIC_SPINDLE_CHECKPOINT	0.7404257	2.398031	0	0	0
PID_AURORA_B_PATHWAY	0.8044711	2.3836968	0	0	0
REACTOME_MITOTIC_METAPHASE_AND_ANAPHASE	0.6831036	2.3595927	0	7.25E-05	0.001
REACTOME_DNA_REPAIR	0.5923209	2.3521383	0	6.69E-05	0.001
REACTOME_SUMOYLATION	0.5919759	2.3408313	0	1.94E-04	0.003
REACTOME_DNA_DOUBLE_STRAND_BREAK_REPAIR	0.66518563	2.3390813	0	1.81E-04	0.003
REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA	0.6771566	2.3287232	0	2.25E-04	0.004
REACTOME_HOMOLOGY_DIRECTED_REPAIR	0.6703193	2.3251	0	2.11E-04	0.004
PID_PLK1_PATHWAY	0.75697136	2.3091893	0	2.00E-04	0.004
REACTOME_HDR_THROUGH_HOMOLOGOUS_RECOMBINATION_HRR	0.72167027	2.3087025	0	1.89E-04	0.004
REACTOME_TRNA_PROCESSING_IN_THE_NUCLEUS	0.7476417	2.3045576	0	1.80E-04	0.004

Table 2 The First 20 Pathways Were Significantly Enriched in GSEA Analysis

NAME	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_FORMATION_OF_FIBRIN_CLOT_CLOTTING_CASCADE	-0.61068314	-1.7498337	0.009689922	0.009689922	0.859
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	-0.5562449	-1.7283566	0	0	0.893
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	-0.5642684	-1.721845	0.00422833	0.00422833	0.907
REACTOME_METABOLISM_OF_ANGIOTENSINOGEN_TO_ANGIOTENSINS	-0.6463841	-1.7085662	0.009451796	0.009451796	0.923
KEGG_ARACHIDONIC_ACID_METABOLISM	-0.5534975	-1.7074896	0.001984127	0.001984127	0.925
REACTOME_BLOOD_GROUP_SYSTEMS_BIOSYNTHESIS	-0.5472932	-1.6724182	0.014056225	0.014056225	0.96
REACTOME_AFLATOXIN_ACTIVATION_AND_DETOXIFICATION	-0.6456393	-1.6684847	0.026369167	0.026369167	0.96
REACTOME_RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA2PLUS	-0.4473624	-1.6665618	0	0	0.963
REACTOME_GLUTATHIONE_CONJUGATION	-0.5390461	-1.6641808	0.03869654	0.03869654	0.966
REACTOME_BIOLOGICAL_OXIDATIONS	-0.40345585	-1.6640171	0.00409836	0.00409836	0.966
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	-0.55111957	-1.6574347	0.026923077	0.026923077	0.971
KEGG_OLFACTORY_TRANSDUCTION	-0.49852335	-1.6479013	0.020242915	0.020242915	0.973
REACTOME_PEROXISOMAL_PROTEIN_IMPORT	-0.4837288	-1.646652	0.02736842	0.02736842	0.973
REACTOME_TNFS_BIND_THEIR_PHYSIOLOGICAL_RECEPTORS	-0.5915594	-1.6268147	0.051923078	0.051923078	0.983
REACTOME_CGMP_EFFECTS	-0.61040384	-1.5627358	0.053537283	0.053537283	0.999
BIOCARTA_INTRINSIC_PATHWAY	-0.5783194	-1.5503914	0.04536862	0.04536862	0.999
REACTOME_ANTIMICROBIAL_PEPTIDES	-0.51218605	-1.5427464	0.04191617	0.04191617	0.999
REACTOME_NITRIC_OXIDE_STIMULATES_GUANYLATE_CYCLASE	-0.5739789	-1.5426029	0.06126482	0.06126482	0.999
REACTOME_PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION	-0.38189727	-1.5331266	0.028248588	0.028248588	0.999

of TNBC by regulating miRNAs, we focused on exploring the miRNA targets of *KIF15*.

KIF15 is one of 5 candidate genes, for regulation of the expression by DNA methylation of facilitator CpGI³⁵ and *KIF15* is associated with severe liver cancer cases and poor prognosis. However, there are no studies on the regulation of *KIF15* by miRNAs. Therefore, the upstream mechanism of *KIF15* deserves additional study. The correlation between patient age and differences in *KIF15* expression indicates that *KIF15* expression may be time-specific. Different TNBC cell lines were originally derived from different tumor patients, and heterogeneity may be the reason for the differences in *KIF15* expression. Additionally, this study demonstrated that augmented gene copy number and decreased DNA methylation levels facilitate gene expression, which is in agreement with the regulatory mechanism of gene expression, ie, changes in gene copy number can increase the gene dose or promote gene expression. Genomic hypomethylation is a common feature of cancer cells, and increasing DNA methylation levels to attenuate the oncogene expression can be used to treat cancer.

Conclusion

The combination of bioinformatics analysis and experiments with the cultured cells identified upstream and downstream regulation of *KIF15*. Our results demonstrated that *KIF15* is a novel oncogene in TNBC and

may be a potential clinical target and biomarker of human malignancy.

Data Sharing Statement

Data and material will be available upon corresponding author approval. All data sets analyzed in this study are included in the manuscript and the additional files.

Ethics Approval and Consent to Participate

Not Applicable

Author Contributions

All authors contributed significantly to concepts and design, data acquisition or data analysis and interpretation; participated in drafting or critical revision of essential academic content; gave final approval to the upcoming version; and agreed to be responsible for all aspects of work. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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