Tumor suppressor syntaxin binding protein 1 regulates alternative splicing of DNA repair genes related to radiation sensitivity in breast cancer cells

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Abstract. As the most aggressive subtype of breast cancer, triple-negative breast cancer (TNBC) is frequently treated with radiotherapy. Downregulation of syntaxin binding protein 1 (STXBP1) gene expression is notably associated with poor prognosis in patients with breast cancer and its protein expression level is associated with tumor radioresistance. However, the precise molecular mechanisms by which STXBP1 regulates breast cancer pathogenesis and radiotherapy resistance remain to be elucidated. In the present study, STXBP1 was overexpressed in MDA-MB-231 cells to investigate its effects on cell proliferation and apoptosis. Transcriptomic sequencing (RNA-sequencing; RNA-seq) was performed to identify differentially expressed genes and alternative splicing events regulated by STXBP1. Additionally, a publicly available RNA-seq dataset (GSE189495) associated with breast cancer radiotherapy, including three fractionally irradiated 20 Gy and three age-matched control MCF-7 cell samples were analyzed to identify radiotherapy-associated alternative splicing alterations. Integrated analysis of these two datasets was conducted to explore the potential mechanisms underlying STXBP1-mediated radiotherapy response in breast cancer. Key gene expression changes and alternative splicing events were validated using reverse transcription-quantitative (RT-q) PCR. In MDA-MB-231 cells, the overexpression of STXBP1 notably inhibited cell proliferation and enhanced levels of apoptosis. Through an analysis of RNA-seq data, the present study discovered that STXBP1 regulates global gene expression and alternative splicing profiles in MDA-MB-231 cells by modulating 111 differentially expressed genes and 1,161 regulated alternative splicing events. STXBP1 plays a key role in regulating the splicing patterns of numerous DNA repair-related genes, including USP48 and PLEC. In addition, the present study conducted an overlap analysis on the transcriptome data from radiotherapy-treated MCF-7 cells alongside the dataset generated in the present study, which revealed 21 alternative splicing events associated with radiotherapy. Notably, these include the DNA repair-related gene UBE2I, and its expression pattern has been confirmed through RT-qPCR. The present study systematically delineated the downstream targets and functional mechanisms of STXBP1 in breast cancer cells, revealing its antitumor molecular role. Moreover, STXBP1 may be associated with radiation sensitivity by regulating the alternative splicing of genes associated with DNA repair. These molecular targets, such as UBE2I, hold potential as novel therapeutic avenues for breast cancer treatment, particularly for TNBC.

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Introduction

Breast cancer is the most prevalent malignant tumor among women (1). In 2020, it emerged as the most common malignant tumor globally, exhibiting the highest incidence rate and ranking fifth among the leading causes of cancer-related mortality worldwide (2). This disease poses a considerable threat to human health. Triple-negative breast cancer (TNBC) is a subtype of breast cancer characterized by the worst prognosis (3). This type of cancer is particularly susceptible to distant metastasis, notably affecting the lungs and brain. TNBC exhibits aggressive invasion and metastatic capabilities, along with a high recurrence rate and mortality (4). TNBC predominantly affects premenopausal women <40 years, representing 15-20% of all breast cancer cases (5). Compared with other subtypes, patients with TNBC experience a shorter survival periods, with a mortality rate of 40% within 5 years following diagnosis (6). Due to the unique characteristics of

TNBC, the absence of estrogen receptors, progesterone receptors and HER2 benefits from conventional hormone therapies and targeted treatments (7). This may represent a notable factor contributing to the poor prognosis observed in patients with TNBC. Consequently, the identification and application of new molecular markers and therapeutic targets are essential for enhancing the therapeutic efficacy of TNBC treatment.

In the management of TNBC, radiotherapy serves as a key treatment modality that can yield optimal long-term outcomes for patients (8). Radiation exerts its effects by directly damaging DNA or inducing single-strand and double-strand breaks (DSBs) through the generation of free radicals (9). In response to radiation exposure, cancer cells activate various signaling pathways, including kinase, survival and metabolic pathways; concurrently, repair enzymes endeavor to mend the damaged DNA. If DNA repair mechanisms fail, this can trigger an apoptosis cascade, ultimately resulting in the death of cancer cells. This process may enhance the survival rate of patients with TNBC (10). Adjuvant radiotherapy following radical surgery for breast cancer offers substantial survival advantages for patients diagnosed with TNBC (11). Furthermore, resistance to radiotherapy is frequently encountered in TNBC (12). Nevertheless, the underlying mechanisms that regulate radiotherapy sensitivity in TNBC remain poorly understood.

Syntaxin binding protein 1 (STXBP1), also referred to as Munc18-1 or SM protein, is a presynaptic membrane-associated protein primarily expressed in the brain, where it regulates soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex-mediated synaptic vesicle docking and fusion (13). Additionally, it plays a vital role in regulating neurotransmitter release. There are also studies suggesting that STXBP1 plays a role in the onset and progression of various types of cancer (14,15). Furthermore, the downregulation of STXBP1 expression is associated with a poor prognosis across various types of cancer (16). Knocking out STXBP1 in human NK cells can substantially impair the cytotoxic activity of NK cells against target cells. The STXBP1/STX1 axis plays a key role in mediating the toxicity of both NK and CD8+ T cells (17). When HOXA11 is inhibited in glioma cells, the efficacy of radiation therapy diminishes. Furthermore, following the knockdown of HOXA11, there is a notable alteration in the expression of STXBP1. This suggests that STXBP1 may be associated with resistance to radiation therapy in glioma (18). However, the precise role and molecular mechanism of STXBP1 in conferring resistance to radiotherapy in TNBC remain unclear.

The present study analyzed the expression and prognostic significance of STXBP1 in breast cancer using public datasets and observed its downregulation in radiation-resistant breast cancer cells. By establishing an STXBP1-overexpressing cell model, cytological experiments and transcriptome sequencing analysis were conducted to investigate its cytological functions and underlying mechanisms. The findings revealed that STXBP1 acts as a potential tumor suppressor gene in breast cancer, likely influencing radiosensitivity of breast cancer cells through regulation of alternative splicing (AS) of DNA damage-related genes. These results provided promising insights for developing future therapeutic strategies for breast cancer, particularly highlighting STXBP1 as a potential target for enhancing radiosensitivity in TNBC radiotherapy.

Materials and methods

Molecular cloning and plasmid preparation. The coding sequence of STXBP1 was cloned into a pcDNA3.1 vector backbone to generate the pcDNA3.1-3xFlag-STXBP1 plasmid, which was procured from Changsha Zeqiong Biotechnology Co., Ltd (RefSeq: NM_001032221). Briefly, the pcDNA3.1 vector was digested with BamHI (cat. no. R0136S; New England Biolabs, Inc.) and XhoI (cat. no. R0146V; New England Biolabs, Inc.) at 37˚C for 2-3 h, and the linearized vector was purified using the MinElute PCR Purification Kit (cat. no. 28004; Qiagen, Inc.). The STXBP1 insert was amplified by PCR and assembled into the vector using the ClonExpress® II One Step Cloning Kit (cat. no. C112; Vazyme Biotech Co., Ltd.) with T4 DNA ligase (cat. no. M0202V; New England Biolabs, Inc.). The ligation products were transformed into competent Escherichia coli cells, and positive clones were selected on LB agar plates containing 100 μg/ml ampicillin (cat. no. 7177-48-2; MilliporeSigma). Positive clones were identified by colony PCR and the insert sequence was verified by Sanger sequencing.

Cell culture and transfections. The human breast cancer cell line MDA-MB-231 (cat. no. CL-0150B; Procell Life Science & Technology Co., Ltd.) was maintained in DMEM medium (cat. no. PM150210; Procell Life Science & Technology Co., Ltd.), supplemented with 10% FBS (FBS; cat. no. 10091148; Gibco; Thermo Fisher Scientific, Inc.), 100 μg/ml streptomycin, 100 U/ml penicillin (cat. no. SV30010; HyClone™; Cytiva). Cells were incubated at 37˚C in a humidified atmosphere containing 5% CO_2. For transient overexpression, transfections were performed with 1 μg of pcDNA3.1-STXBP1 plasmid (Changsha Zeqiong Biotechnology Co., Ltd.) or the empty pcDNA3.1 vector per well in 12-well plates using Lipofectamine® 3000 reagent (cat. no. L3000015; Invitrogen; Thermo Fisher Scientific, Inc.), strictly adhering to the manufacturer's instructions. MDA-MB-231 cells transfected with the empty pcDNA3.1 vector served as the negative control (NC) group. Cells were harvested 48 h post-transfection for subsequent reverse transcription-quantitative (RT-q) PCR and western blotting.

RT-qPCR. Total RNA was extracted from cells seeded at $2x10^5$ per well in 12-well plates and harvested 48 h post-transfection using TRIzol® reagent (cat. no. 15596-018; Ambion; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was reverse-transcribed into cDNA using a commercial reverse transcription kit (cat. no. R323-01; Vazyme Biotech Co., Ltd.) according to the manufacturer's instructions. The reaction conditions were set as follows: 42˚C for 5 min, 37˚C for 15 min and 85˚C for 5 sec using a Bio-Rad T100 thermal cycler. qPCR was then performed on the ABI QuantStudio 5 system using SYBR Green Master Mix (cat. no. 11202ES08; Vazyme Biotech Co., Ltd.) according to the manufacturer's protocol with the following cycling parameters: Initial denaturation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for

1 min. All samples were run in technical triplicates. The $2^{-\Delta\Delta Cq}$ method was applied to calculate relative mRNA expression levels, using GAPDH as the endogenous control for normalization (19). Primer sequences used for qPCR analysis are listed in Table SI. All qPCR assays were performed with three independent biological replicates and each biological replicate was run in three technical replicates.

Western blot analysis. To prepare protein lysates, MDA-MB-231 cells were resuspended in ice-cold RIPA lysis buffer (cat. no. PR20001; Proteintech Group, Inc.) containing a protease inhibitor cocktail (cat. no. 4693116001; MilliporeSigma) and incubated on ice for 30 min. Protein concentrations were determined using a BCA protein assay, and equal amounts of protein (25 μg per lane) were loaded. Lysates were then mixed with protein loading buffer (cat. no. P1040; Beijing Solarbio Science & Technology Co., Ltd.), boiled for 10 min and subjected to electrophoresis on 10% SDS-PAGE gels. Subsequently, proteins were transferred onto 0.45 μm PVDF membranes (cat. no. ISEQ00010; MilliporeSigma). After blocking with 5% non-fat milk for 1 h at room temperature, the membranes were incubated overnight at 4˚C with primary antibody against the STXBP1 (cat. no. 67137-1-Ig; 1:1,000; Proteintech Group, Inc.) and GAPDH (Mouse anti-GAPDH; cat. no. 60004-1-AP 1:5,000; Proteintech Group, Inc.). Following primary antibody incubation, membranes were probed with an HRP-conjugated anti-mouse secondary antibody (cat. no. AS003; 1:10,000; ABclonal Biotech Co., Ltd.) for 45 min at room temperature. Protein bands were visualized using the enhanced chemiluminescence detection reagent (cat. no. P0018FM; Beyotime Biotechnology). Densitometric analysis was performed using ImageJ software (v1.53; National Institutes of Health).

Cell proliferation assay. Cell proliferation was assessed using a Cell Counting kit-8 (CCK-8; cat. no. HY-K0301; MedChemExpress). Briefly, MDA-MB-231 cells were seeded in 24-well plates at a density of 35,000 cells per well. Control wells received an equal volume of PBS and blank wells contained culture medium only. After 24 h of culture, 10 μl of CCK-8 solution was added to each well, followed by a 3-h incubation period. Absorbance was then measured at 450 nm using a microplate reader (cat. no. ELX800; Biotek; Agilent Technologies, Inc.). The cell proliferation rate was calculated using the formula: Proliferation rate (%)=[(OD_experimental - OD_blank)/(OD_control - OD_blank)] x100%.

Apoptosis detection by flow cytometry. The level of apoptosis was evaluated using an Annexin V-Alexa Fluor647/PI apoptosis detection kit (cat. no. 40304ES60; Shanghai Yeasen Biotechnology Co., Ltd.), following the supplier's protocol. MDA-MB-231 cells were plated in six-well plates, transfected with the STXBP1 plasmid after 48 h of culture at 37˚C and compared with PBS-treated control cells. Cells were then harvested, resuspended in binding buffer, and stained with 5 μl Annexin V and 10 μl propidium iodide (PI, 20 μg/ml). After incubating for 10-15 min at room temperature in the dark, samples were immediately analyzed by flow cytometry (FACSCanto; BD Biosciences). The apoptotic rate was calculated as the percentage of early (Annexin V^+/PI^-) plus late (Annexin V^+/PI^+) apoptotic cells. Data were analyzed using CytExpert software (v2.0; Beckman Coulter, Inc.).

RNA extraction and sequencing (RNA-seq). Total RNA was isolated from cells using TRIzol® reagent (cat. no. 15596-018; Ambion; Thermo Fisher Scientific, Inc.). To ensure high purity, the RNA underwent two rounds of phenol-chloroform extraction and was subsequently treated with RQ1 DNase (cat. no. M6101; Promega Corporation) to eliminate genomic DNA contamination. RNA concentration and purity were assessed by measuring the A260/A280 ratio on an Implen N50 Touch spectrophotometer. RNA integrity was further confirmed by 1.0% agarose gel electrophoresis.

RNA-seq library construction and sequencing were conducted by Wuhan Ruixing Biotechnology Co., Ltd. Briefly, 1 μg of DNase-treated total RNA was used for directional library preparation with the VAHTS® Universal V8 RNA-seq Library Prep Kit for Illumina (cat. no. NR605; Vazyme Biotech Co., Ltd.). mRNA was enriched using VAHTS mRNA capture beads (cat. no. N401; Vazyme Biotech Co., Ltd.). The purified mRNA was fragmented and reverse-transcribed into double-stranded cDNA. Following end repair, A-tailing and adapter ligation (using VAHTS RNA Multiplex Oligos Set 1 for Illumina; cat. no. N323; Vazyme Biotech Co., Ltd.), the libraries were amplified by PCR, purified, quantified and stored at -80˚C until sequencing. The use of dUTP during second-strand synthesis enabled strand-specific sequencing. Library quality was verified by agarose gel electrophoresis, showing main fragments between 300-500 bp. Final libraries were quantified by Qubit fluorometry and diluted to a loading concentration of 5.76 pM on an Illumina Novaseq 6000 platform (Illumina Inc.) for 150 bp paired-end reads.

Public sequencing data analysis. Breast cancer gene-level expression data (comprising 1,118 tumor and 103 normal samples) were downloaded from Genomic Data Commons (GDC; https://portal.gdc.cancer.gov). Raw sequencing data related to breast cancer radiotherapy were downloaded from the Gene Expression Omnibus (GEO) under accession number GSE189495 (20). This dataset included three fractionally irradiated 20 Gy (FIR20) and three age-matched control (AMC) MCF-7 cell samples.

RNA-seq raw data cleaning and alignment. Raw sequencing reads were subjected to quality control. Reads containing >2 ambiguous (N) bases were discarded. Adapter sequences and low-quality bases were then trimmed using the FASTX-Toolkit (v.0.0.13; http://hannonlab.cshl.edu/fastx_toolkit/). Reads <16 nt after trimming were also excluded. The resulting high-quality clean reads were aligned to the human reference genome (GRCh38) using HISAT2 (v.2.2.1; https://daehwankimlab.github.io/hisat2), permitting up to four mismatches (21). Uniquely mapped reads were retained for subsequent gene-level quantification and fragments per kilobase of transcript per million fragments mapped calculation (22).

Differentially expressed genes (DEGs) analysis. DEGs analysis between experimental groups was performed using the DESeq2 (23) package in R/Bioconductor (v.4.3.4;

https://bioconductor.org/). Genes with an adjusted P-value (Benjamini-Hochberg) (24) of <0.05 and an absolute fold change >2 ($|\log_2 FoldChange|>1$) were classified as statistically significant DEGs.

AS analysis. The AS events (ASEs) and regulated ASEs (RASEs) were identified and quantified using the ABLas pipeline (25,26). The tool detects 10 types of ASEs based on splice junction reads, including exon skipping (ES), alternative 5' splice site (A5SS), alternative 3' splice site (A3SS), mutually exclusive exons (MXE), mutually exclusive 5' UTRs (5pMXE), mutually exclusive 3' UTRs (3pMXE), cassette exon, A3SS and ES and A5SS and ES.

To define STXBP1-regulated ASEs, a Student's t-test was applied to compare the ratio of AS events between groups (unpaired two-tailed t-test). Events passing a false discovery rate (FDR) cut-off of 5% were considered markedly regulated.

Functional enrichment analysis. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses for DEGs and genes harboring RASEs (RASGs) were conducted using the KOBAS 2.0 server (27). Significance of enrichment was determined via a hypergeometric test, with the Benjamini-Hochberg procedure applied to control the FDR.

PPI network and module analysis of DEGs. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/) was used to assess the interacting partners of the DEGs. Visualization of the network was performed with Cytoscape (version 3.6.1; https://cytoscape.org/) software. After constructing the PPI network, topology characteristics of the network were analyzed using the Network Analyzer plug-in of Cytoscape software, and degree distribution, distribution of the shortest path, average clustering coefficient and closeness centrality were examined. Subsequently, the top 10 significant hub genes or key nodes were identified according to their connectivity degrees by using the CytoHubba tool, a plug-in for Cytoscape. The MCODE plug-in (version 2.0.3; https://apps.cytoscape.org/apps/mcode) was used to analyze the highly interconnected clusters of this network with default parameters.

Prognostic analysis. Prognostic information for genes of interest in TNBC was retrieved from the Kaplan-Meier Plotter online database (http://kmplot.com/analysis).

Expression of STXBP1 in The Cancer Genome Atlas (TCGA). The STXBP1 expression data (1,085 tumor samples and 291 normal samples) was downloaded from GEPIA (http://GEPIA 2. cancer-pku.cn/).

Statistical analysis. Student's t-test was performed to evaluate the differences between the STXBP1-overexpression (OE-STXBP1) and control (OE-NC) groups (unpaired two-tailed t-test). For comparisons involving three or more groups, one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference post hoc test was performed to correct for multiple comparisons. The results from each experiment were presented as mean ± SEM based on three independent replicates. Data analysis was carried out using SPSS 23.0 software (IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

STXBP1 downregulation in breast cancer and post-radiotherapy is associated with improved prognosis. To investigate the expression changes of STXBP1 in breast cancer cells following radiotherapy, RNA-seq data (GSE189495) from MCF-7 breast cancer cells without (AMC; n=3) or with fractional irradiation (FIR20; n=3) were downloaded and analyzed. The fractionally irradiated MCF-7 cells (FIR20) exhibited a profile of radioresistance, whereas the AMC group was radiosensitive (20). The results demonstrated that STXBP1 mRNA levels were markedly elevated in radiation-sensitive breast cancer cells (AMC) compared with radiation-resistant cells (FIR20; Fig. 1A), suggesting that STXBP1 may be involved in regulating radiosensitivity in breast cancer. Furthermore, the expression profile of STXBP1 in patients with TNBC was analyzed using the online tool GEPIA 2 (http://gepia.cancer-pku.cn/). By categorizing TCGA samples into normal (n=291) and primary tumor (n=1,085) groups, the present study observed that STXBP1 was significantly downregulated in tumor tissues compared with normal tissues (P<0.05; Fig. 1B). Subgroup analysis of primary tumors based on clinical stages revealed consistently lower STXBP1 expression across all stages of breast cancer when compared with normal samples (Fig. 1C). To validate these findings at the protein level, data from the Clinical Proteomic Tumor Analysis Consortium (https://ualcan.path.uab.edu/index.html) was incorporated. Analysis revealed a significant reduction in STXBP1 protein expression in patients with breast cancer (Fig. 1D and E), with a more pronounced downregulation observed in the TNBC subtype (Fig. 1F). Integrative analysis of RNA and protein data demonstrated a concordant trend of STXBP1 suppression in breast cancer. Additionally, Kaplan-Meier (https://kmplot.com/analysis/) survival analysis using relapse-free survival as the endpoint revealed a significant association between higher STXBP1 expression and improved patient prognosis (Fig. 1G). Collectively, STXBP1 exhibited significant downregulation in breast cancer tissues, with its expression level showing a positive correlation with patient prognosis. Furthermore, this molecule is highly expressed in radiation-sensitive cells, indicating its potential as a candidate target for enhancing radiosensitivity in radiotherapy.

STXBP1 overexpression reprograms the global transcriptome in MDA-MB-231 cells. Given the low expression of STXBP1 observed in both breast cancer tissues and radiation-treated breast cancer cells, as well as its reduced expression across various types of cancer that are associated with poor prognosis (28), it was hypothesized that STXBP1 may modulate malignant phenotypes and regulate downstream effector molecules. To test this, an STXBP1-overexpressing model (OE STXBP1) in MDA-MB-231 cells was established by transfection with the pIRES-hrGFP-1a-STXBP1 recombinant plasmid, while the control group (NC) was transfected with

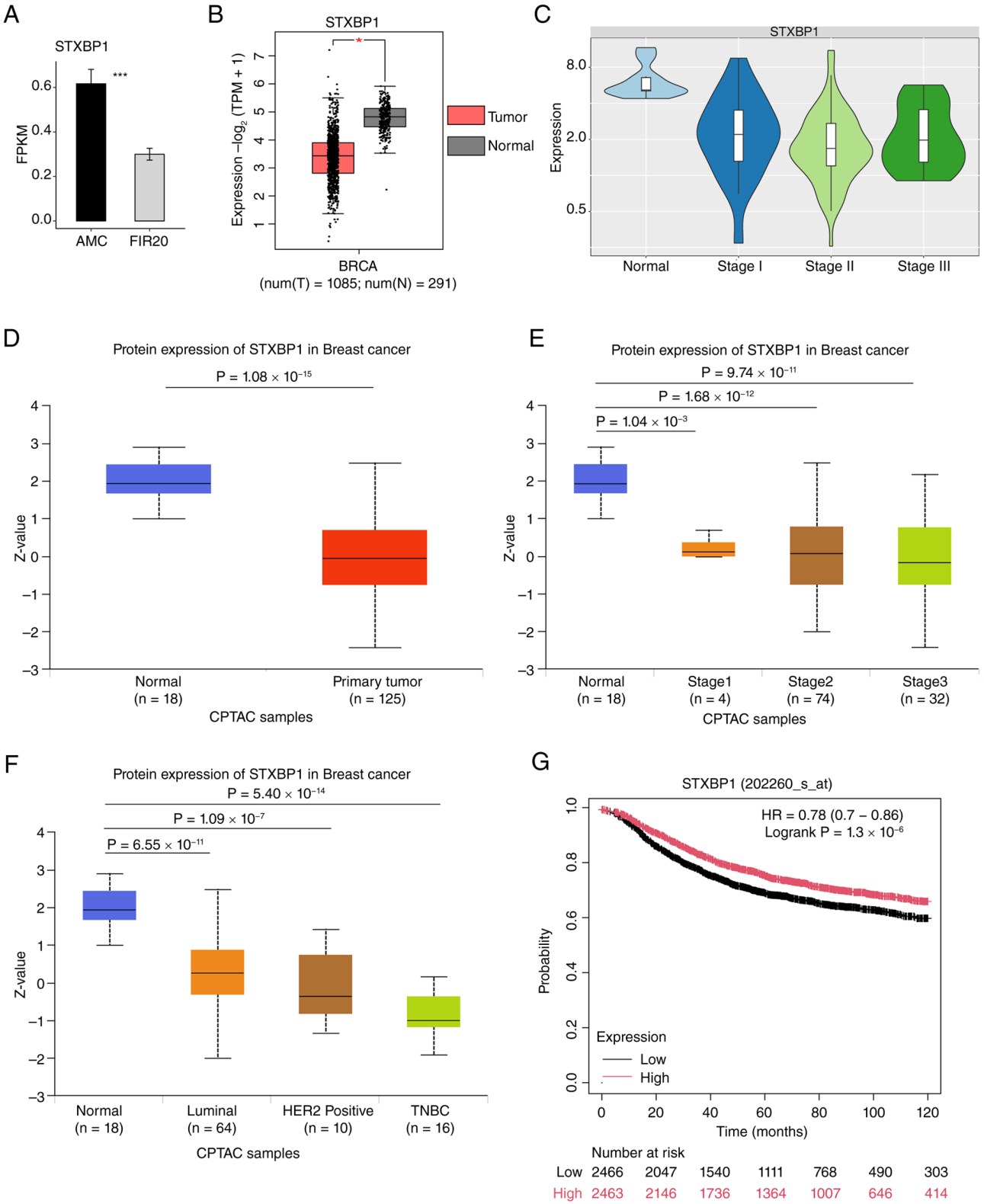

Figure 1. Suppressed STXBP1 expression in breast cancer and irradiated breast cancer cells. (A) Bar plot illustrating the expression pattern and statistical difference of STXBP1. (B) The expression level of STXBP1 in TCGA-BRCA data set. (C) Violin plot showing the expression level of STXBP1 in different tumor stage samples (data downloaded from TCGA). (D) Box plot showing the decreased protein level of STXBP1 in breast cancer samples. (E) Box plot showing the decreased protein level of STXBP1 in breast cancer samples separated by different stages. (F) Box plot showing the decreased protein level of STXBP1 in breast cancer samples separated by breast cancer subtype. (G) Line plot showing the significant association between recurrence free survival time and STXBP1 expression in patients with breast cancer with high/low expression. Error bars represent mean ± SEM. *P<0.05, ***P<0.001. STXBP1, syntaxin binding protein 1; TCGA, The Cancer Genome Atlas.

the empty vector. RT-qPCR confirmed a significant increase in STXBP1 mRNA levels in the transfected group compared with controls (P<0.001; Fig. 2A), while western blot analysis validated synchronous upregulation of STXBP1 protein

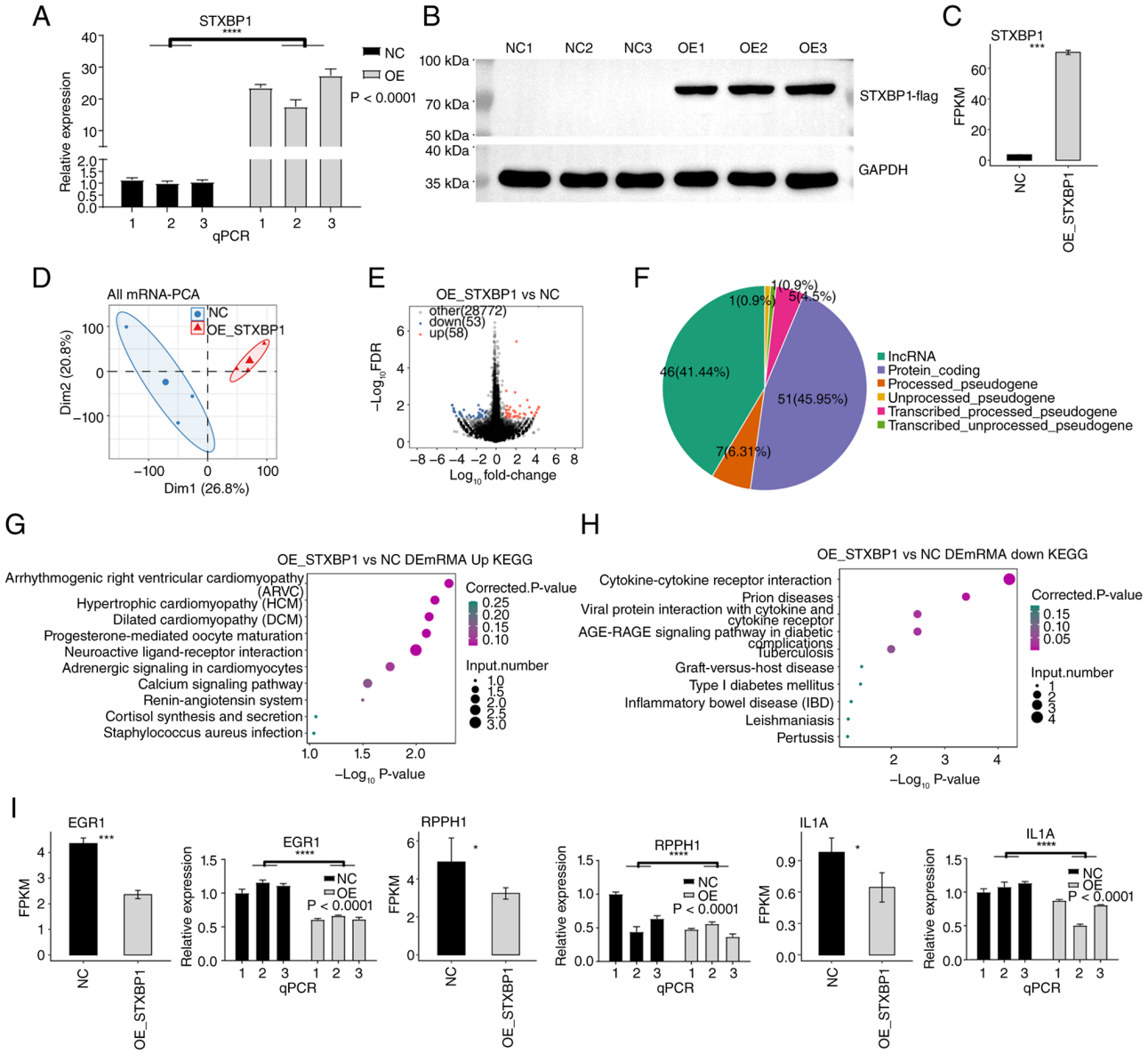

Figure 2. STXBP1 regulates gene expression in MDA-MB-231 cells. (A) Histogram displaying RT-qPCR results for NC and OE samples. (B) Western blot analysis confirmed successful STXBP1 overexpression. (C) Bar plot depicting STXBP1 expression levels based on RNA-seq data. (D) PCA performed using FPKM values of all detected genes in NC and OE STXBP1 samples. (E) Volcano plot illustrating all DEGs identified between OE STXBP1 and NC groups. (F) Pie chart exploring the ratio of gene type in DEGs. (G) Scatter plot highlighting the most markedly enriched KEGG pathways for upregulated DEGs. (H) Scatter plot highlighting the most markedly enriched KEGG pathways for downregulated DEGs. (I) Left: Bar plot presenting the expression pattern and statistical difference of DEGs. Right: RT-qPCR validation of corresponding gene expression levels. Error bars represent mean ± SEM. *P<0.05, ***P<0.001, ****P<0.0001. STXBP1-overexpression group vs. NC group. STXBP1, syntaxin binding protein 1; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; OE, overexpressed; RNA-seq, RNA-sequencing; PCA, principal component analysis; FPKM, fragments per kilobase million; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

(Fig. 2B). These results confirmed the successful establishment of an STXBP1-OE MDA-MB-231 model.

Functional assays revealed that while STXBP1 overexpression did not markedly induce apoptosis, it demonstrated a pro-apoptotic tendency (Fig. S1A and B). The proliferation rate of OE STXBP1 samples was markedly inhibited (Fig. S1C). Concurrently, these findings suggested that STXBP1 may exert tumor-suppressive effects through both anti-proliferative and pro-apoptotic mechanisms.

To elucidate molecular mechanisms, transcriptomic sequencing on OE STXBP1 (n=3) and NC (n=3) samples was performed. Following the acquisition of high-quality sequencing reads and their comparison with the human genome, the present study confirmed robust STXBP1 upregulation in the OE group (Fig. 2C). Principal component analysis conducted on the total expressed genes revealed distinct separation of transcriptional profiles between groups (Fig. 2D), indicating STXBP1-mediated reprogramming of the cellular transcriptome. Differential expression analysis identified 111 markedly altered genes (58 upregulated; 53 downregulated), with volcanic plots visualizing their distribution (Fig. 2E; Table SII). Gene type analysis revealed predominant enrichment of protein-coding genes and long non-coding RNAs (lncRNAs), suggesting STXBP1 may regulate disease

progression through coordinated lncRNA and mRNA modulation (Fig. 2F). Functional enrichment analysis of DEGs showed significant enrichment of upregulated genes in metabolic pathways and disease-related processes, while downregulated genes clustered in cytokine-cytokine receptor interaction and viral protein interaction with cytokine and cytokine receptor (Fig. 2G and H). Validation via RT-qPCR confirmed that the expression patterns of three breast cancer-related genes (EGR1, RPPH1 and IL1A) were downregulated following STXBP1 overexpression, consistent with the sequencing data (Fig. 2I). Survival analysis revealed a strong association between IL1A overexpression and poor prognosis in patients with TNBC (Fig. S2), further supporting the tumor-suppressive role of STXBP1 through regulation of key effector molecules. Collectively, these findings demonstrated a significant association between STXBP1 dysfunction and altered expression of tumor-suppressive genes in MDA-MB-231 cells. The data suggested that STXBP1 plays a pivotal role in suppressing cancer progression through transcriptional reprogramming, positioning it as a potential therapeutic target for breast cancer intervention.

STXBP1 modulates AS of DNA damage repair genes in MDA-MB-231 cells. To further elucidate the function of STXBP1, the present study systematically investigated its regulatory role in AS events in MDA-MB-231 cells. A total of 59,645 known AS events and 78,172 novel AS events were identified. Statistical analysis revealed significantly different splicing patterns between OE STXBP1 and NC groups ($P<0.05$), indicating that STXBP1 overexpression induces global disruption of the AS landscape (Tables SIII and IV). These AS events encompassed 88 cassette exons, 26 mutually exclusive exons (MXE), 403 intron retentions (Intron R), 78 ES, 34 alternative 5' splice site events with ES (A5SS and ES), 197 alternative 5' splicing sites (A5SS), 25 alternative 3' splice site events with ES (A3SS and ES), 208 alternative 3' splicing sites (A3SS), 44 alternative 5' mutually exclusive UTRs (5pMXE) and 58 alternative 3' mutually exclusive UTRs (3pMXE; Fig. 3A). Among these, Intron R, A5SS and A3SS constituted the predominant splicing types (Fig. 3A; Tables SV and SVI), highlighting the distinctive regulatory feature of STXBP1 on the AS profile. Functional enrichment analysis demonstrated that genes harboring STXBP1-regulated AS events were predominantly enriched in biological processes associated with DNA repair, negative regulation of DNA template transcription, cellular responses to DNA damage stimulus and double-strand break repair (Fig. 3B). These pathway alterations may underlie the observed proliferation inhibition in OE STXBP1 group. Notably, negative regulation of apoptosis was also markedly enriched in RASGs (Fig. 3B), potentially explaining the elevated apoptosis levels in OE STXBP1 samples. To further validate the regulatory role of STXBP1 in the AS patterns of DNA damage repair genes, the present study designed specific primers capable of distinguishing between canonical splice sites and alternative splice sites and performed RT-qPCR validation for USP48 and PLEC. Experimental results confirmed that STXBP1 overexpression markedly upregulated PLEC A3SS splicing ratio while downregulating USP48 A3SS splicing ratio, consistent with RNA-seq data (Fig. 3C and D). Collectively, these findings demonstrated that STXBP1 critically modulates the AS landscape, particularly in DNA damage repair genes, potentially contributing to its tumor-suppressive functions.

STXBP1 regulated AS genes for DNA repair are also markedly enriched in breast cancer radiation cells. Hierarchical clustering analysis was performed using the radiotherapy-related RNA-seq dataset (GSE189495) from the GEO database. The results indicated high intra-group consistency among the three replicates of radiation-exposed breast cancer cell samples (FIR20) and control cell samples (AMC; Fig. 4A). Subsequent investigation of AS regulation in irradiated cells identified 1,628 significant radiotherapy-associated RASEs, comprising 26 3pMXEs, 38 5pMXEs, 108 A3SS, 8 A3SS and ES, 16 A5SS and ES, 167 A5SS, 62 ES, 23 MXEs, 93 cassette Exons and 1,087 intronR events (Fig. 4B). Among these, Intron R, A5SS and A3SS were the predominant regulatory types (Fig 4B). Further functional enrichment analysis revealed that genes associated with these differentially spliced events were markedly enriched in DNA repair and cellular responses to DNA damage stimuli (Fig. 4C and D), a finding consistent with the AS events influenced by STXBP1 overexpression. These findings suggest that RASEs may modulate radiotherapy sensitivity in breast cancer cells by regulating DNA damage repair mechanisms.

To elucidate the specific mechanism of STXBP1 in radiotherapy, an overlap analysis between OE STXBP1 and radiotherapy-induced splicing events in breast cancer was conducted, identifying 21 co-regulated genes (Fig. 4E). Further employing protein-protein interaction (PPI) network analysis, DLG1, GOLGA3, HDLBP, EED, ORC3, RNF34, CHFR, SAMD4B, PLD3 and UBE2I as core genes from the 21 candidates were identified (Fig. 4F). This indicates that STXBP1 may function by coordinately regulating AS levels of key genes. Notably, the A5SS event of UBE2I, a gene closely involved in DNA damage repair, was downregulated following STXBP1 overexpression, with RT-qPCR validation results consistent with RNA-seq data. In irradiated breast cancer cells, overexpression of STXBP1 resulted in a decreased ratio of UBE2I A5SS splicing events (Fig. 4G). Survival analysis using Kaplan-Meier Plotter revealed a significant positive correlation between UBE2I expression and prognosis in patients with TNBC patients who exhibited increased UBE2I expression was associated with improved survival outcomes (Fig. S2).

To summarize, these findings suggested that STXBP1 may participate in radiotherapy resistance mechanisms in TNBC by reshaping the AS spectrum of breast cancer cells post-radiotherapy, particularly through modulating splicing patterns of DNA damage repair-related genes. Targeting splicing regulation of key genes such as UBE2I could provide a novel therapeutic strategy to improve prognosis in patients with TNBC.

Discussion

Previous research has demonstrated that reduced expression of STXBP1 is associated with cancer progression across various solid tumors in humans (16). Nevertheless, the molecular mechanisms by which STXBP1 contributes to TNBC progression

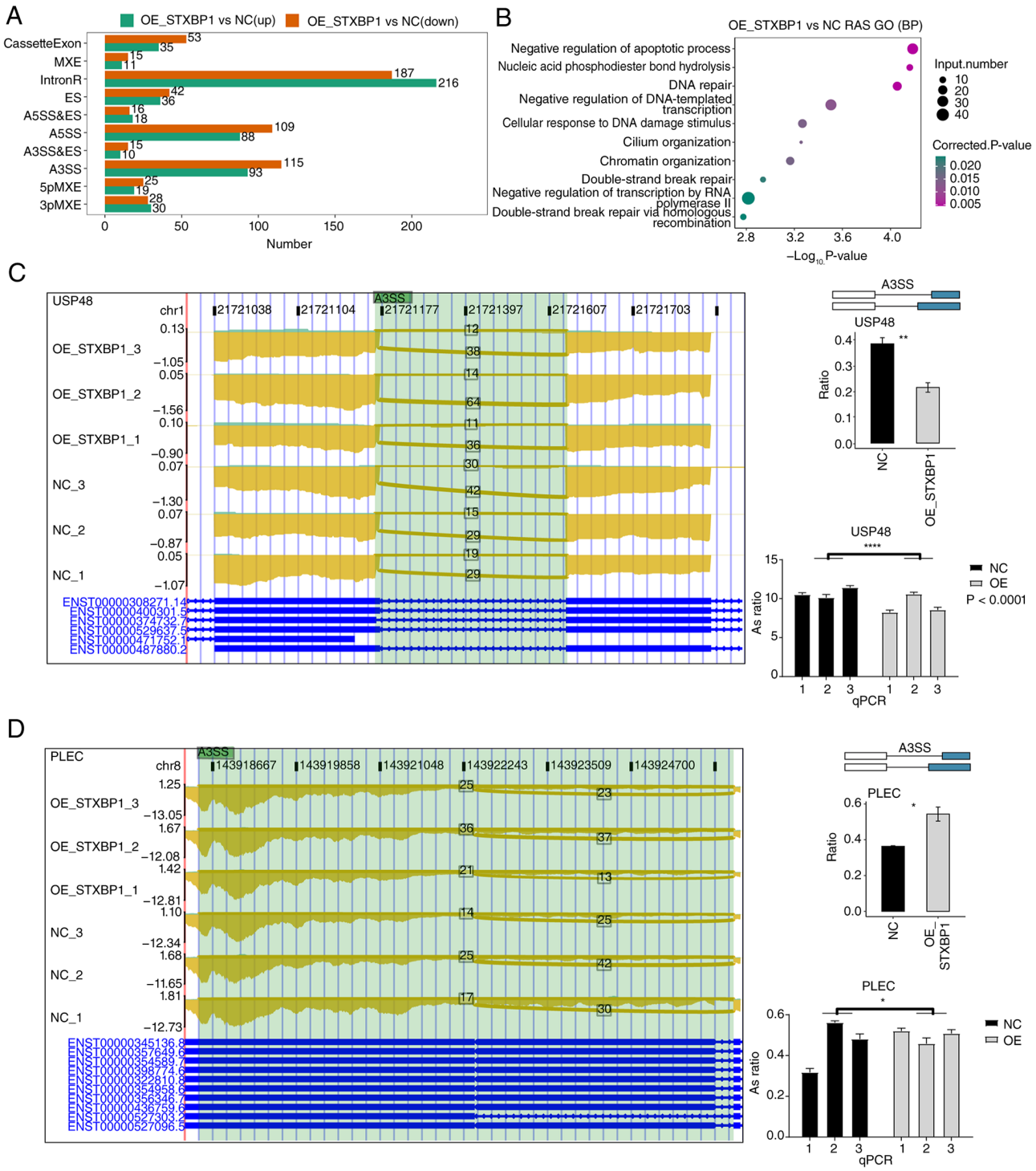

Figure 3. STXBP1 influences the AS of genes in MDA-MB-231 cells. (A) Bar plot illustrating STXBP1-regulated AS events. Y-axis: Distinct AS event types. (B) Scatter plot displaying the most enriched GO biological process terms among the regulated AS genes. (C) STXBP1 modulates USP48 AS. Left panel: IGV-sashimi plot depicting regulated AS events and binding sites along the mRNA. Upper subpanel: Read distribution of RASEs; lower subpanel: Gene transcript structures. Right panel: Schematic diagrams showing ASE architectures. Bottom of right panel: RNA-seq quantification and RT-qPCR validation of ASEs. (D) STXBP1 modulates PLEC AS. Left panel: IGV-sashimi plot depicting regulated AS events and binding sites along the mRNA. Upper subpanel: Read distribution of RASEs; lower subpanel: gene transcript structures. Right panel: Schematic diagrams showing ASE architectures. Bottom of right panel: RNA-seq quantification and RT-qPCR validation of ASEs. Error bars represent mean ± SEM. *P<0.05, **P<0.01, ****P<0.0001. STXBP1-overexpression group vs. NC group. STXBP1, syntaxin binding protein 1; AS, alternative splicing; ASE, AS events; RASE, regulated ASEs; RNA-seq, RNA-sequencing RT-qPCR, reverse transcription-quantitative PCR.

remain poorly understood. The present study systematically investigated the regulatory effects of STXBP1 on intracellular DEGs and RASEs within MDA-MB-231 cells, as well as its potential regulatory functions in the context of breast cancer radiotherapy. The present findings indicated that STXBP1 expression levels were decreased in patients with breast cancer and in radiation-resistant breast cancer cells and that STXBP1 overexpression inhibited cell proliferation. RNA-seq analysis

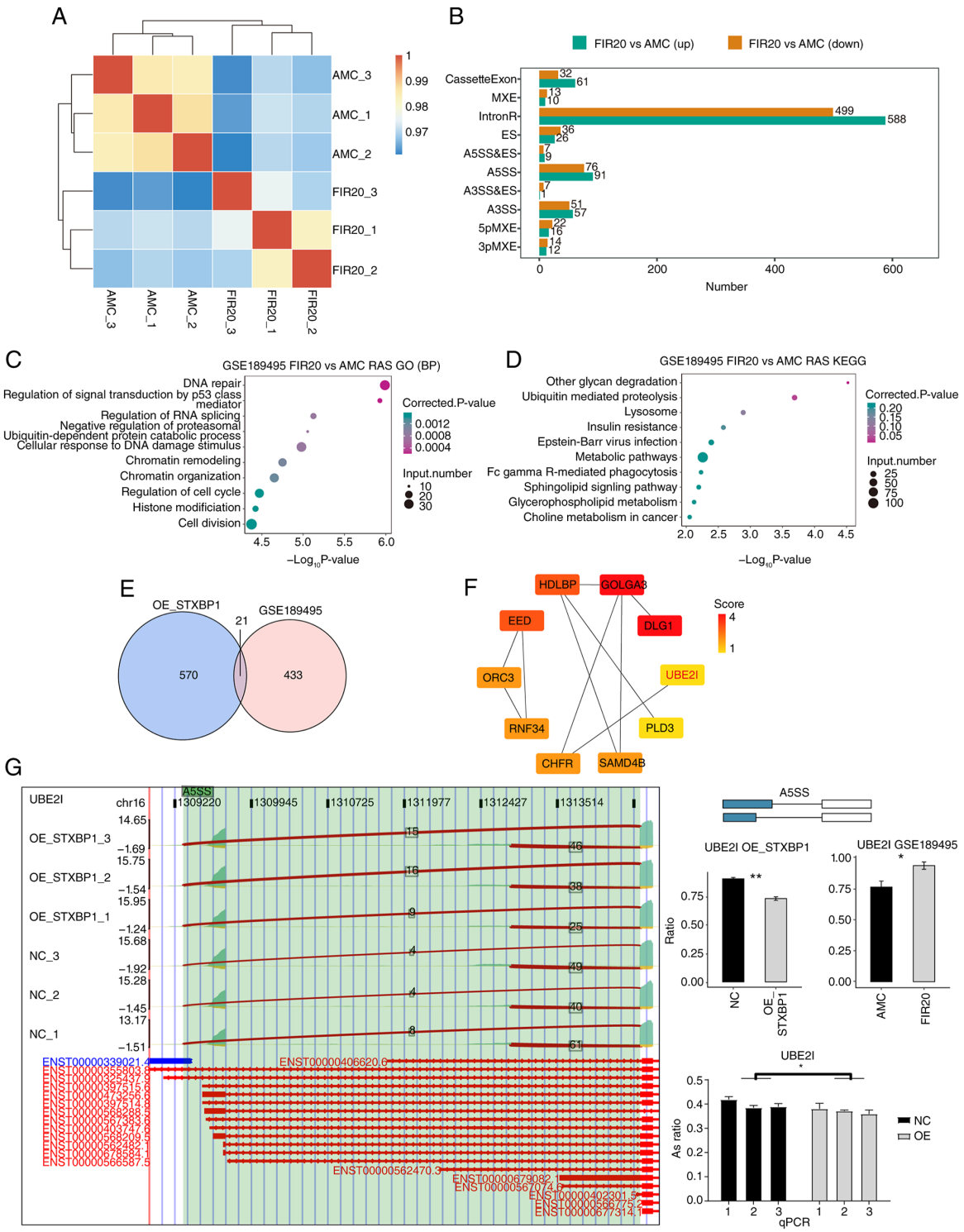

Figure 4. Verification of key target genes of STXBP1. (A) Correlation plot showing the relativity of all samples in GSE189495. (B) Bar plot showing the RASE in GSE189495. (C) The scatter plot exhibiting the most enriched GO biological process results of the RASG in GSE189495. (D) The scatter plot exhibiting the most enriched KEGG results of the RASG in GSE189495. (E) Venn diagram showing the overlapped RASE between OE STXBP1 vs. NC and FIR20 vs. AMC (data from GSE189495). (F) Hub genes identified by PPI analysis. (G) STXBP1 modulates UBE2I AS. Left panel: IGV-sashimi plot depicting regulated AS events and binding sites along the mRNA. Upper subpanel: Read distribution of RASEs; lower subpanel: Gene transcript structures. Right panel: Schematic diagrams showing ASE architectures. Bottom of right panel: RNA-seq quantification and RT-qPCR validation of ASEs. Error bars represent mean ± SEM. *P<0.05, **P<0.01. STXBP1, syntaxin binding protein 1; RASE, regulated alternative splicing events; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; RASG, genes harboring RASEs; OE, overexpressed; NC, negative control; FIR20, fractionally irradiated 20 Gy; PPI, protein-protein interaction; AS, alternative splicing.

confirmed that STXBP1 overexpression influenced the expression of multiple genes and AS patterns. Functional analysis indicated that STXBP1 and radiotherapy-affected RASGs are both involved in DNA damage and repair pathways. The present study proposed that STXBP1 may exert an important function in breast cancer through regulating DNA repair

processes and the expression of AS-related genes, thus potentially representing a key target for radiotherapy. The present study underscores the potential regulatory value of STXBP1 in breast cancer and identifies downstream RNA molecules that may serve as novel therapeutic targets for future breast cancer treatments.

Literature reviews indicate that low expression of STXBP1 is associated with poor prognosis in multiple malignant tumors (28,29). However, its biological functions and molecular regulatory mechanisms in breast cancer, particularly in TNBC, remain under-investigated. Through clinical sample analysis, the present study revealed that STXBP1 exhibits markedly reduced expression in breast cancer tissues, with more pronounced downregulation observed in the TNBC subtype. Functional experiments demonstrated that STXBP1 overexpression markedly suppresses the proliferative capacity of MDA-MB-231 cells without markedly inducing apoptosis. These findings suggested that STXBP1 may primarily exert its tumor-suppressive effects by modulating proliferation-related pathways rather than apoptotic pathways.

Transcriptome sequencing revealed that STXBP1 overexpression led to differential expression of 111 genes (58 upregulated; 53 downregulated). Notably, downregulated genes showed significant enrichment in pathways such as cytokine-cytokine receptor interactions and virus protein-cytokine/cytokine receptor interactions. Previous studies have established that cytokines and their receptors interactions critically influence survival of patients with cancer, while potential functional genetic variations within the genes associated with these pathways may impact the survival rates of patients with TNBC (30,31). As a key regulatory mechanism for tumor cell proliferation and metastasis, cytokine and cytokine receptor signaling pathways were previously confirmed as core mediators of migration, invasion and metastasis in MDA-MB-231 cells (32). Among the downregulated genes under OE STXBP1 conditions, EGR1, RPPH1 and IL1A exhibited significant expression changes. Early growth response 1 (EGR1) is often dysregulated in various types of cancer, exhibiting both tumor inhibitory and promoter activities. In breast cancer tissues, the expression of EGR1 is downregulated and this downregulation associated positively with a lower survival rate among patients with breast cancer (33). RPPH1, identified as a tumor promoter in breast cancer cells, enhances the proliferation of breast cancer cells and facilitates cell cycle progression by downregulating micro RNA (miR)-122 (34). IL1A is a key inflammatory cytokine that plays a considerable role in tumor invasion, angiogenesis and metastasis by inducing the expression of VEGF, TNF-α, and TGF-β (35). In addition, the present findings indicated that high IL1A expression in patients with breast cancer associates with worse survival outcomes. The aforementioned results suggested that STXBP1 may influence breast cancer progression by modulating the expression of these cytokines. However, due to the current lack of systematic exploration regarding the interactions between STXBP1 and cytokines/their receptors, further experimental validation is still required to support the conclusions of the present study.

Previous studies have confirmed that dysregulation of AS is involved in the occurrence and development of breast cancer, affecting multiple biological processes such as cell proliferation, apoptosis, invasion and metastasis (36-38). The present study found that STXBP1 overexpression markedly altered 1,161 AS events. The functionally enriched spliced genes were involved in biological processes including apoptotic process, DNA repair, cellular response to DNA damage stimulus and double-strand break repair. These results suggest that STXBP1 may inhibit breast cancer progression by regulating the DNA damage response. The accumulation of DNA damage serves as a core driver of carcinogenesis, manifesting through induction of key gene mutations (for example, activation of proto-oncogenes such as RAS and MYC or inactivation of tumor suppressor genes including TP53, BRCA1 and BRCA2) and chromosomal instability arising from unresolved DSBs, such as chromosomal translocations, telomere shortening and epigenetic dysregulation (for example, aberrant DNA methylation) (39-41). These processes collectively fuel tumor heterogeneity and malignant progression. Further analysis revealed significant alterations in splicing events of USP48 (A3SS) and PLEC (A3SS) upon STXBP1 overexpression. USP48, a deubiquitinating enzyme that regulates homologous recombination repair, inhibits BRCA1-mediated DNA repair by deubiquitinating histone H2A (42). Furthermore, SNRPA1 regulates the AS of exon 31 of PLEC, resulting in alterations in the proportion of expressed lectin isoforms and subsequently influencing the metastatic potential of breast cancer cells (43). Collectively, these findings indicated that STXBP1 may play a regulatory role in the progression of TNBC by influencing these key AS events. Future studies should further explore the regulatory mechanisms of AS in genes involved in signaling pathways such as DNA damage and repair. Such research may provide an important theoretical foundation for the development of novel therapeutic strategies for malignant tumors.

The initial step in radiation response involves DNA damage (44). Radiotherapy-induced DNA damage types include base damage, single-strand breaks, DSBs and interstrand crosslinks. Among these, unrepaired or improperly repaired DSBs represent the key lesion type mediating ionizing radiation-induced cell death (45-47). Notably, a previous study has indicated that STXBP1 may play a role in the development of resistance to radiation therapy in glioma (18). In the field of breast cancer, the mechanisms underlying STXBP1-mediated radioresistance remain to be fully elucidated. The present study analyzed GEO breast cancer radiation cell samples and discovered that STXBP1 exhibits low expression levels in these samples. Furthermore, 1,628 significant RASEs with significant regulatory features were identified in post-radiotherapy breast cancer cell samples. Functional enrichment analysis revealed these RASEs were predominantly enriched in pathways related to DNA repair, p53 signal transduction, RNA splicing regulation and cellular responses to DNA damage. This result showed substantial overlap with the pathways enriched by splicing events regulated by STXBP1 overexpression, suggesting that STXBP1 may influence radioresistant phenotypes by modulating AS of DNA damage-related genes.

Cross-referencing analysis identified 21 AS genes commonly involved in both datasets. PPI network analysis screened 10 core genes: DLG1, GOLGA3, HDLBP, EED, ORC3, RNF34, CHFR, SAMD4B, PLD3 and UBE2I. Among these, UBE2I is associated with proliferation regulation and apoptosis resistance in breast cancer cells following radiotherapy exposure. As a member of the E2 enzyme family,

dysregulated UBE2I has been associated with unfavorable prognosis in hepatocellular carcinoma, glioblastoma and prostate cancer (48-50). A previous study confirmed that the splicing factor SF3B1 plays a key role in myelodysplastic syndromes by mediating AS of UBE2I. Additionally, miR-10a-5p-mediated post-transcriptional regulation of UBE2I is involved in cervical cancer initiation, invasion and metastasis (51). Clinical data analysis demonstrated a positive association between high UBE2I expression and improved prognosis in patients with TNBC. Notably, the ratio of UBE2I AS increased markedly in post-radiotherapy breast cancer cells, while STXBP1 overexpression reversed this phenomenon. These findings suggested that STXBP1 may participate in mediating radiotherapy resistance in TNBC by regulating AS of UBE2I. These discoveries provide potential intervention targets for enhancing radiotherapy sensitivity in TNBC. Therefore, future studies could be conducted to validate these findings using MDA-MB-231 radiotherapy cell models and TNBC radiotherapy tissue samples, and extend the investigations to other breast cancer cell lines. Concurrently, gain- and loss-of-function experiments should be planned to systematically dissect the specific mechanisms of STXBP1-related AS in the development of radiotherapy resistance in TNBC.

Several limitations of the present study should be acknowledged. First, all functional experiments and splicing analyses were performed exclusively in the MDA-MB-231 triple-negative breast cancer cell line. Whether STXBP1 regulates AS in a similar manner in other TNBC lines (for example, BT-549 and MDA-MB-468) or in non-TNBC subtypes (for example, luminal A/B and HER2-positive) remains unknown and requires further investigation. Second, the radiotherapy-associated transcriptomic data (GSE189495) were derived from MCF-7 cells, which are ER-positive and HER2-low, whereas the STXBP1 overexpression experiments were conducted in MDA-MB-231 (TNBC) cells. These distinct cellular contexts limit the direct comparability of the two datasets. The cross-analysis is therefore exploratory and hypothesis-generating, rather than confirmatory. The overlapping genes identified (such as UBE2I) represent potential molecular nodes of interest, but the underlying regulatory mechanisms may differ across breast cancer subtypes. Third, the present study did not directly manipulate STXBP1 expression in irradiated cells or perform radiosensitivity assays (for example, clonogenic survival after irradiation) in the STXBP1-overexpressing model. Future studies should address these gaps by using isogenic cell lines, conducting gain- and loss-of-function experiments under radiation conditions and validating findings in patients with TNBC-derived samples or clinical cohorts.

In summary, the present study systematically analyzed the regulatory role of STXBP1 in breast cancer cells and radiation resistance. The findings indicated that STXBP1 regulates the enrichment pathways of DEGs and AS genes that are associated with the inhibition of tumor-promoting characteristics in STXBP1-overexpressing samples. These pathways primarily involve DNA repair signaling and cellular responses to DNA damage stimuli. Therefore, it was hypothesized that STXBP1 may play a role in the progression of TNBC by regulating the AS of genes associated with the DNA damage repair pathway. Furthermore, the present study concluded that it is implicated in the biological mechanisms underlying radiation therapy for TNBC. The present study proposes that STXBP1 may play a key role in regulating breast cancer progression by modulating the expression and AS patterns of specific genes. This regulation could unveil new molecular targets and therapeutic strategies for future breast cancer treatments, particularly aimed at enhancing the sensitivity of TNBC to radiation therapy, based on the regulatory network associated with STXBP1.

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Availability of data and materials

The data generated in the present study may be found in the NCBI GEO database under accession number GSE287393 or at the following URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE287393&token=kxwbkkwmvfgpzqn.

Authors' contributions

MZ and FC conceived and designed the study. FC reviewed and edited the manuscript. MZ drafted the original manuscript. YL participated in study design and manuscript revision. ML and QR performed experiments. HW performed bioinformatics analysis. AA performed experiments and statistical analysis. XZ contributed to data interpretation and manuscript editing. MZ and FC confirm the authenticity of all the raw data. All authors have read and approved 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.

References

1. Xiong X, Zheng LW, Ding Y, Chen YF, Cai YW, Wang LP, Huang L, Liu CC, Shao ZM and Yu KD: Breast cancer: Pathogenesis and treatments. Signal Transduct Target Ther 10: 49, 2025.

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
3. Li Y, Zhang H, Merkher Y, Chen L, Liu N, Leonov S and Chen Y: Recent advances in therapeutic strategies for triple-negative breast cancer. J Hematol Oncol 15: 121, 2022.
4. Zagami P and Carey LA: Triple negative breast cancer: Pitfalls and progress. NPJ Breast Cancer 8: 95, 2022.
5. Lee J: Current treatment landscape for early triple-negative breast cancer (TNBC). J Clin Med 12: 1524, 2023.
6. Tong L, Yu X, Wang S, Chen L and Wu Y: Research progress on molecular subtyping and modern treatment of triple-negative breast cancer. Breast Cancer (Dove Med Press) 15: 647-658, 2023.
7. Guo R, Luo X, Liu J, Liu L, Wang X and Lu H: Omics strategies decipher therapeutic discoveries of traditional Chinese medicine against different diseases at multiple layers molecular-level. Pharmacol Res 152: 104627, 2020.
8. Upadhyay R and Bazan JG: Advances in radiotherapy for breast cancer. Surg Oncol Clin N Am 32: 515-536, 2023.
9. Huang RX and Zhou PK: DNA damage response signaling pathways and targets for radiotherapy sensitization in cancer. Signal Transduct Target Ther 5: 60, 2020.
10. Johnson J, Chow Z, Napier D, Lee E, Weiss HL, Evers BM and Rychahou P: Targeting PI3K and AMPKalpha signaling alone or in combination to enhance radiosensitivity of triple negative breast cancer. Cells 9: 1253, 2020.
11. Yao Y, Chu Y, Xu B, Hu Q and Song Q: Radiotherapy after surgery has significant survival benefits for patients with triple-negative breast cancer. Cancer Med 8: 554-563, 2019.
12. Ding L, Gu H, Xiong X, Ao H, Cao J, Lin W, Yu M, Lin J and Cui Q: MicroRNAs involved in carcinogenesis, prognosis, therapeutic resistance and applications in human triple-negative breast cancer. Cells 8: 1492, 2019.
13. André T, van Berkel AA, Singh G, Abualrous ET, Diwan GD, Schmenger T, Braun L, Malsam J, Toonen RF, Freund C, *et al*: Reduced protein stability of 11 pathogenic missense STXBP1/MUNC18-1 variants and improved disease prediction. Biol Psychiatry 96: 125-136, 2024.
14. Wang X, Fu G, Wen J, Chen H, Zhang B and Zhu D: Membrane location of syntaxin-binding protein 1 is correlated with poor prognosis of lung adenocarcinoma. Tohoku J Exp Med 250: 263-270, 2020.
15. Zhang W, Qu H, Ma X, Li L, Wei Y, Wang Y, Zeng R, Nie Y, Zhang C, Yin K, *et al*: Identification of cuproptosis and immune-related gene prognostic signature in lung adenocarcinoma. Front Immunol 14: 1179742, 2023.
16. Yu M, Tian Y, Wu M, Gao J, Wang Y, Liu F, Sheng S, Huo S and Bai J: A comparison of mRNA and circRNA expression between squamous cell carcinoma and adenocarcinoma of the lungs. Genet Mol Biol 43: e20200054, 2020.
17. Lopez JA, Noori T, Minson A, Li Jovanoska L, Thia K, Hildebrand MS, Akhlaghi H, Darcy PK, Kershaw MH, Brown NJ, *et al*: Bi-Allelic mutations in STXBP2 reveal a complementary role for STXBP1 in cytotoxic lymphocyte killing. Front Immunol 9: 529, 2018.
18. Se YB, Kim SH, Kim JY, Kim JE, Dho YS, Kim JW, Kim YH, Woo HG, Kim SH, Kang SH, *et al*: Underexpression of HOXA11 is associated with treatment resistance and poor prognosis in glioblastoma. Cancer Res Treat 49: 387-398, 2017.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
20. Inalegwu A, Cuypers B, Claesen J, Janssen A, Coolkens A, Baatout S, Laukens K, De Vos WH and Quintens R: Fractionated irradiation of MCF7 breast cancer cells rewires a gene regulatory circuit towards a treatment-resistant stemness phenotype. Mol Oncol 16: 3410-3435, 2022.
21. Kim D, Langmead B and Salzberg SL: HISAT: A fast spliced aligner with low memory requirements. Nat Methods 12: 357-360, 2015.
22. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ and Pachter L: Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 28: 511-515, 2010.
23. Love MI, Huber W and Anders S: Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15: 550, 2014.
24. Benjamini Y and Hochberg Y: Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Statist Soc B 57: 289-300, 1995.
25. Jin L, Li G, Yu D, Huang W, Cheng C, Liao S, Wu Q and Zhang Y: Transcriptome analysis reveals the complexity of alternative splicing regulation in the fungus Verticillium dahliae. BMC Genomics 18: 130, 2017.
26. Xia H, Chen D, Wu Q, Wu G, Zhou Y, Zhang Y and Zhang L: CELF1 preferentially binds to exon-intron boundary and regulates alternative splicing in HeLa cells. Biochim Biophys Acta Gene Regul Mech 1860: 911-921, 2017.
27. Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li CY and Wei L: KOBAS 2.0: A web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res 39(Web Server Issue): W316-W322, 2011.
28. Liu H, Zhang J, Wei C, Liu Z, Zhou W, Yang P, Gong Y and Zhao Y: Prognostic signature construction of energy metabolism-related genes in pancreatic cancer. Front Oncol 12: 917897, 2022.
29. Wang Z, Sun X, Gao L, Guo X, Feng C, Lian W, Deng K and Xing B: Comprehensive identification of a two-genesignature as a novel potential prognostic model for patients with medulloblastoma. Am J Transl Res 12: 1600-1613, 2020.
30. Chan SH, Kuo WH and Wang LH: SCEL regulates switches between pro-survival and apoptosis of the TNF-α/TNFR1/NF-κB/c-FLIP axis to control lung colonization of triple negative breast cancer. J Biomed Sci 30: 93, 2023.
31. Gao L, Chen S, Hong M, Zhou W, Wang B, Qiu J, Xia J, Zhao P, Fu L, Wang J, *et al*: Kinectin 1 promotes the growth of triple-negative breast cancer via directly co-activating NF-kappaB/p65 and enhancing its transcriptional activity. Signal Transduct Target Ther 6: 250, 2021.
32. Messeha SS, Zarmouh NO, Asiri A and Soliman KFA: Rosmarinic acid-induced apoptosis and cell cycle arrest in triple-negative breast cancer cells. Eur J Pharmacol 885: 173419, 2020.
33. Saha SK, Islam SMR, Saha T, Nishat A, Biswas PK, Gil M, Nkenyereye L, El-Sappagh S, Islam MS and Cho SG: Prognostic role of EGR1 in breast cancer: A systematic review. BMB Rep 54: 497-504, 2021.
34. Zhang Y and Tang L: Inhibition of breast cancer cell proliferation and tumorigenesis by long non-coding RNA RPPH1 down-regulation of miR-122 expression. Cancer Cell Int 17: 109, 2017.
35. You D, Jeong Y, Yoon SY, Kim SA, Lo E, Kim SW, Lee JE, Nam SJ and Kim S: Entelon(®) (Vitis vinifera Seed Extract) prevents cancer metastasis via the downregulation of interleukin-1 alpha in triple-negative breast cancer cells. Molecules 26: 3644, 2021.
36. Deng L, Liao L, Zhang YL, Yang SY, Hu SY, Andriani L, Ling YX, Ma XY, Zhang FL, Shao ZM and Li DQ: SF3A2 promotes progression and cisplatin resistance in triple-negative breast cancer via alternative splicing of MKRN1. Sci Adv 10: eadj4009, 2024.
37. Yang Q, Zhao J, Zhang W, Chen D and Wang Y: Aberrant alternative splicing in breast cancer. J Mol Cell Biol 11: 920-929, 2019.
38. Wang N, Shi B, Ding L, Zhang X, Ma X, Guo S, Qiao X, Wang L, Ma D and Cao J: FMRP protects breast cancer cells from ferroptosis by promoting SLC7A11 alternative splicing through interacting with hnRNPM. Redox Biol 77: 103382, 2024.
39. He C, Kawaguchi K and Toi M: DNA damage repair functions and targeted treatment in breast cancer. Breast Cancer 27: 355-362, 2020.
40. Mirza-Aghazadeh-Attari M, Recio MJ, Darband SG, Kaviani M, Safa A, Mihanfar A, Sadighparvar S, Karimian A, Alemi F, Majidinia M and Yousefi B: DNA damage response and breast cancer development: Possible therapeutic applications of ATR, ATM, PARP, BRCA1 inhibition. DNA Repair (Amst) 98: 103032, 2021.
41. He X, Li Y, Li J, Li Y, Chen S, Yan X, Xie Z, Du J, Chen G, Song J and Mei Q: HDAC2-Mediated METTL3 delactylation promotes DNA damage repair and chemotherapy resistance in triple-negative breast cancer. Adv Sci (Weinh) 12: e2413121, 2025.
42. Velimezi G, Robinson-Garcia L, Muñoz-Martínez F, Wiegant WW, Ferreira da Silva J, Owusu M, Moder M, Wiedner M, Rosenthal SB, Fisch KM, *et al*: Map of synthetic rescue interactions for the Fanconi anemia DNA repair pathway identifies USP48. Nat Commun 9: 2280, 2018.
43. Fish L, Khoroshkin M, Navickas A, Garcia K, Culbertson B, Hänisch B, Zhang S, Nguyen HCB, Soto LM, Dermit M, *et al*: A prometastatic splicing program regulated by SNRPA1 interactions with structured RNA elements. Science 372: eabc7531, 2021.

44. Edwards DM, Speers C and Wahl DR: Targeting noncanonical regulators of the DNA damage response to selectively overcome cancer radiation resistance. Semin Radiat Oncol 32: 64-75, 2022.
45. Anderson CJ, Talmane L, Luft J, Connelly J, Nicholson MD, Verburg JC, Pich O, Campbell S, Giaisi M, Wei PC, *et al*: Strand-resolved mutagenicity of DNA damage and repair. Nature 630: 744-751, 2024.
46. Melia E and Parsons JL: DNA damage and repair dependencies of ionising radiation modalities. Biosci Rep 43: BSR20222586, 2023.
47. Strzałka W, Zgłobicki P, Kowalska E, Bażant A, Dziga D and Banaś AK: The dark side of UV-Induced DNA lesion repair. Genes (Basel) 11: 1450, 2020.
48. Zhang Y, Jiang F, He H, Ye J, Mao X, Guo Q, Wu SL, Zhong W, Wu CL and Lin N: Identification of a novel microRNA-mRNA regulatory biomodule in human prostate cancer. Cell Death Dis 9: 301, 2018.
49. Yang L, Sun HF, Guo LQ and Cao HB: MiR-10a-5p: A promising biomarker for early diagnosis and prognosis evaluation of bladder cancer. Cancer Manag Res 13: 7841-7850, 2021.
50. Li X and Meng Y: SUMOylation regulator-related molecules can be used as prognostic biomarkers for glioblastoma. Front Cell Dev Biol 9: 658856, 2021.
51. Gu Y, Feng X, Jin Y, Liu Y, Zeng L, Zhou D and Feng Y: Upregulation of miRNA-10a-5p promotes tumor progression in cervical cancer by suppressing UBE2I signaling. J Obstet Gynaecol 43: 2171283, 2023.