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The role of splicing factor *PRPF8* in breast cancer

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Abstract.
BACKGROUND: Alternative splicing is a mechanism to produce different proteins with diverse functions from one gene. Many splicing factors play an important role in cancer progression. *PRPF8* is a core protein component of the spliceosome complex, U4/U6-U5 tri-snRNP.
OBJECTIVE: However, *PRPF8* involved in mRNA alternative splicing are rarely included in the prognosis.
METHODS: We found that *PRPF8* was expressed in all examined cancer types. Further analyses found that *PRPF8* expression was significantly different between the breast cancer and paracancerous tissues.
RESULTS: Survival analyses showed that *PRPF8*-high patients had a poor prognosis, and the expression of *PRPF8* is associated with distant metastasis-free survival (DMFS) and post progression survival (PPS). Gene Set Enrichment Analysis (GSEA) has revealed that *PRPF8* expression is correlated with TGF-β, JAK-STAT, and cell cycle control pathways. Consistent with these results, upon *PRPF8* silencing, the growth of MCF-7 cells was reduced, the ability of cell clone formation was weakened, and $p21$ expression was increased.
CONCLUSIONS: These results have revealed that *PRPF8* is a significant factor for splicing in breast cancer progression.

Keywords: PRPF8, breast cancer, alternative splicing, prognosis

1. Introduction

Breast cancer is a common cancer, which has a higher morbidity rate in women [1]. The clinical outcomes of breast cancer are closely linked to prognostic parameters, such as tumor size, grade, and lymph node and metastasis status. However, genes involved in mRNA alternative splicing are rarely included in the prognosis. By generating multiple mRNAs from a precursor mRNA (pre-mRNA), alternative splicing greatly diversify the genome coding capacity. Most genes are multiple-exon genes and generate more than one functional protein.

Recent studies have confirmed multiple splicing factors affect the splicing of critical breast cancer-related genes [2]. *PRPF8* is a core protein component of the spliceosome complex, U4/U6-U5 tri-snRNP and contains several WD repeats, which function in protein-protein interactions. It participates in the two sequential transesterification steps of pre-mRNAs during the cut and link of pre-mRNAs. Loss of *PRPF8* can lead to the death of mouse embryonic cells [3] and Drosophila cells [4]. Thus, the study

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Table 1
The shRNA sequences

Oligo	Sequence
PRPF8-1	TCACGTAACACATACAGGG
PRPF8-4	ACAACACAAGCACAGACAG
Control	TTACTCTCGCCCAAGCGAG

aimed to investigate the significance of *PRPF8* in breast cancer. Our present study, the data of Oncomine and TCGA (The Cancer Genome Atlas) were used to analyze the expression levels of *PRPF8* in normal tissue and carcinomas. The clinical significance of *PRPF8* in breast cancer was further explored. GSEA analysis and *in vitro* experiments have disclosed the possible role of *PRPF8* in breast cancer.

2. Materials and methods

2.1. Bioinformatic and statistical analysis

The expression of *PRPF8* in different cancer types, between cancer samples and adjacent tissues, is analyzed from GEO and Oncomine database (https://www.oncomine.org/). P values less than 1×10^{-4} were considered statistically significant. UALCAN (http://ualcan.path.uab.edu/index.html) dataset was used to analyze the *PRPF8* protein levels in breast cancer tissue. Km-plot (https://kmplot.com) and TCGA portal (http://www.tumorsurvival.org/index.html) websites were used to analyze the expression of *PRPF8* in breast cancer survival. The GSEA (http://www.broadinstitute.org/gsea/index.jsp) analyze the expression level of *PRPF8* was used as the phenotype label, and "Metric for ranking genes" was set to Pearson Correlation. The downstream pathways (positive and negative) of *PRPF8* are obtained by calculating the enrichment score of data in the breast cancer group ($n = 1106$). The enrichment score, and multiple test corrections reveal the biological characteristics and genetic regulatory network of *PRPF8*.

2.2. Cell culture

MCF-7 and HEK-293T cells were obtained from the National Infrastructure of Cell Line Resource. MCF-7 cells were cultured in α-MEM medium containing 10% fetal bovine serum (FBS) and 10 μg/ml insulin. HEK-293T cells were cultured in DMEM medium containing 10% FBS. All the cells were cultured at 37°C.

2.3. RNA purification and quantitative reverse transcription-polymerase chain reaction

Viral packaging vectors of pMD2 (Addgene, USA) and pPAX2 (Addgene, USA) and pGIPZ (Addgene, USA) were transfected into 293T cells with Lipofectamine 2000. The shRNA sequences were listed (Table 1), for pGIPZ. After 3 days, the medium was purified with 0.45 μm filters and mixed with 1/3 volumes of lentiviral concentration solution. After the mixture solution was incubated at 4°C for 12 h, it was centrifuged at 4°C for 45 min, and pellets were resuspended in PBS. 100 μl viruses were added to 2×10^5 MCF-7 cells, and the harvested cells were used to extract total RNA. Reverse transcription of RNA with PrimeScript RT kit (Takara, Japan). Quantitative PCR (q-PCR) Detection System using iTaq Universal SYBR Green Supermix. Primers for q-PCR were listed (Table 2). Data were analyzed using the $2^{\Delta\Delta Ct}$ method.

Table 2
Primers used for quantitative PCR

Primer	Sequence
PRPF8-qF	TGTCAGTTGCGTGTCTTCAT
PRPF8-qR	AGACAGTAAAACTCCCATCA
P21-qF	TGTCTTGTACCCTTGTGCCT
P21-qR	AAGATGTAGAGCGGGCCTTT

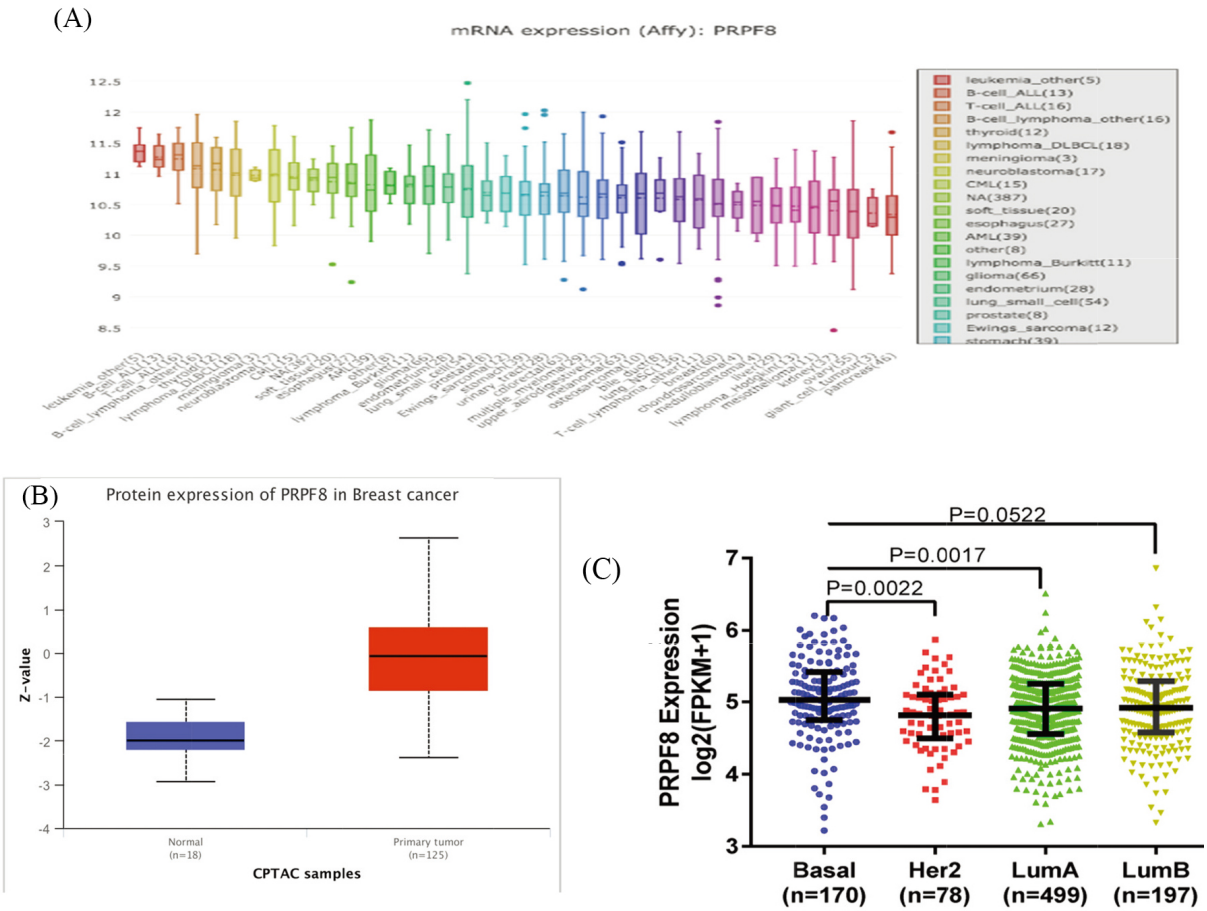

Fig. 1. (A) The mRNA expression of *PRPF8* in different cancer cell lines. (B) The protein levels of *PRPF8* are higher in breast cancer than in adjacent normal tissue. (C) *PRPF8* expression in the subtypes of breast cancer.

2.4. Colony formation and cell proliferation

500 MCF-7 cells per well were grown in six-well plates and maintained at 37°C for 12 days. The medium was replaced every 3 days. After 12 days, the cells were stained with crystal violet for 20 minutes as described in the product manual. The colonies were counted and subject to statistical analyses. In the cell proliferation analysis, the same cells were seeded in 96-well plates and the CCK-8 kits were used to detect cell viability on day 1, 3, 5. Data were normalized to day 1 and presented as mean ± standard deviation.

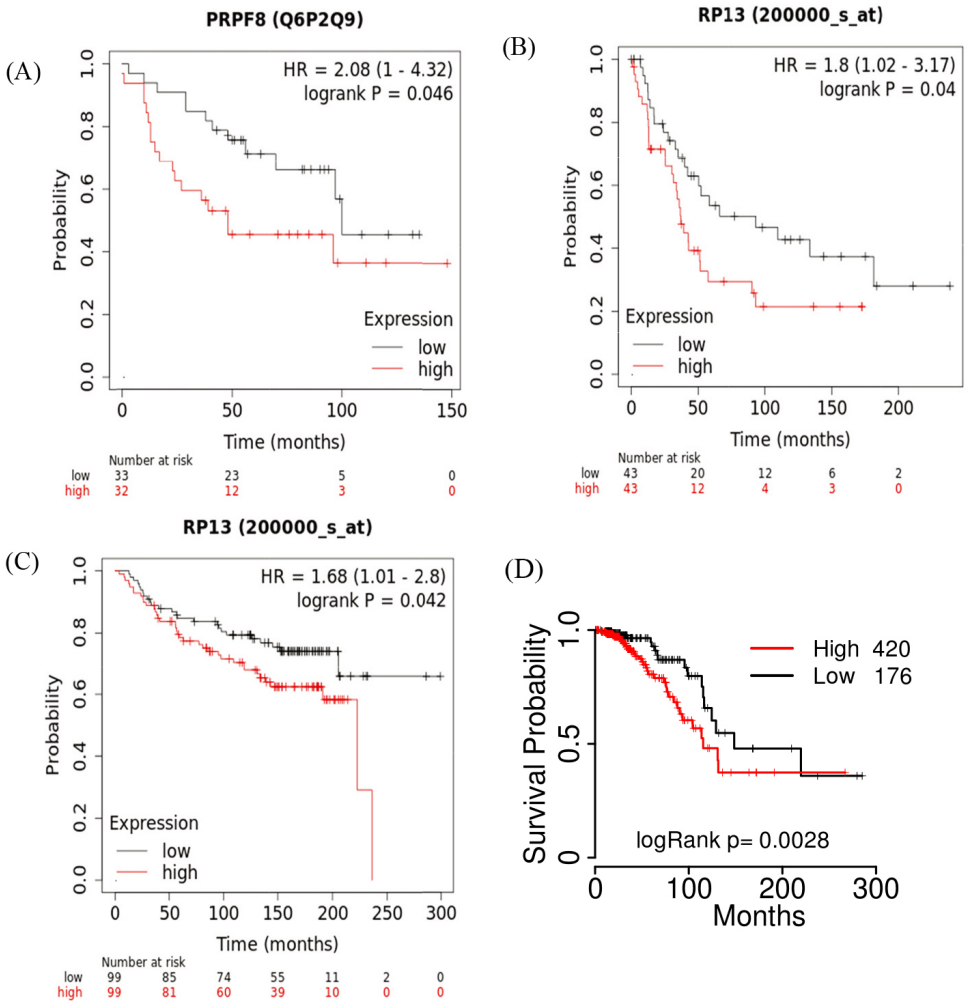

Fig. 2. (A) *PRPF8* protein levels are associated with the OS of breast cancer patients in Kaplan-Meier plots. (B) The mRNA expression of *PRPF8* is associated with the DMFS probabilities of breast patients. (C) The mRNA expression of *PRPF8* is associated with the PPS probabilities of breast patients. (D) Prognostic analysis of *PRPF8* with mRNA expression in luminal A patients.

3. Results

3.1. Characterizing the expression of PRPF8 in breast cancer

There are few reports on the expression of *PRPF8* in breast cancer. In this study we analyzed the expression levels of *PRPF8* in various human tumors from the Oncomine database and Cancer Cell Line Encyclopedia (CCLE) (fold change of > 4, gene rank of $> 10\%$, and p value $< 1 \times 10^{-4}$ was set as the threshold). *PRPF8* is expressed in all subtypes of cancers (Fig. 1A). Then we compared *PRPF8* expression between normal samples ($n = 18$) and primary breast tumor samples ($n = 125$) in UALCAN database (http://ualcan.path.uab.edu/index.html), and has found *PRPF8* protein expression is significantly higher in primary tumors (Fig. 1B). (*PRPF8* proteomic expression profile based on sample types, and Z-values represent standard deviations from the median across samples. p value $= 4.3\text{E}^{-14}$).

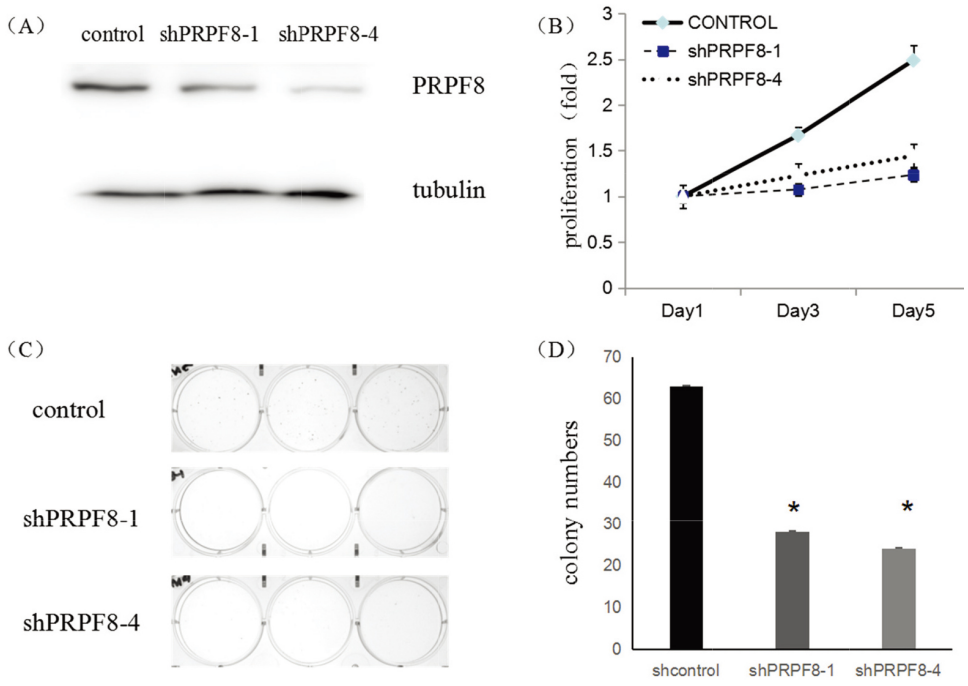

Fig. 3. (A) WB shown the inhibition of *PRPF8* shRNA in MCF-7 cells. (B) MCF-7 cells growth curves by transduced with *PRPF8* different shRNAs. (C) The images of MCF-7 colony formation, which were transduced with different shRNAs. (D) The numbers of colonies after MCF-7 cells were transduced with different shRNAs.

3.2. PRPF8 expression is associated with the survival of breast cancer patients

Subsequently, we investigated the association of *PRPF8* expression with breast cancer patient survival. In breast cancer, the *PRPF8* protein level was associated with the overall survival (OS) according to Kaplan-Meier plots database. Consistent with this result, *PRPF8* mRNA expression is also associated with the OS in the Kaplan-Meier plots cohort. In the cohort of (GSE7390), we also found that *PRPF8* expression was associated with the PPS ($p < 0.05$, HR $= 1.68$), and DMFS ($p < 0.05$, HR $= 1.8$) (Fig. 2). To further characterize the role of *PRPF8* in breast cancer, we analyzed the association of *PRPF8* expression with the patient OS in different subtypes of breast cancer using the TCGA dataset. The results have shown that *PRPF8* expression is significantly associated with the OS in Luminal A patients, but not in other subtypes of breast cancer, indicating *PRPF8* might play a role in Luminal A type cancer (Fig. 2D).

3.3. Inhibition of PRPF8 expression impaired breast cancer cell proliferation

To confirm the function of the *PRPF8* in breast cancer, we employed shRNAs to silence *PRPF8* expression in breast cancer cells, MCF-7. Western Blot (WB) shown that both shRNAs suppressed the expression of *PRPF8* (Fig. 3A). Meanwhile, inhibiting *PRPF8* expression, the numbers of colonies decreased (Fig. 3C and D), and the growth curves were significantly inhibited, suggesting that *PRPF8* was essential for the maintenance of the proliferation in cancer cells (Fig. 3B).

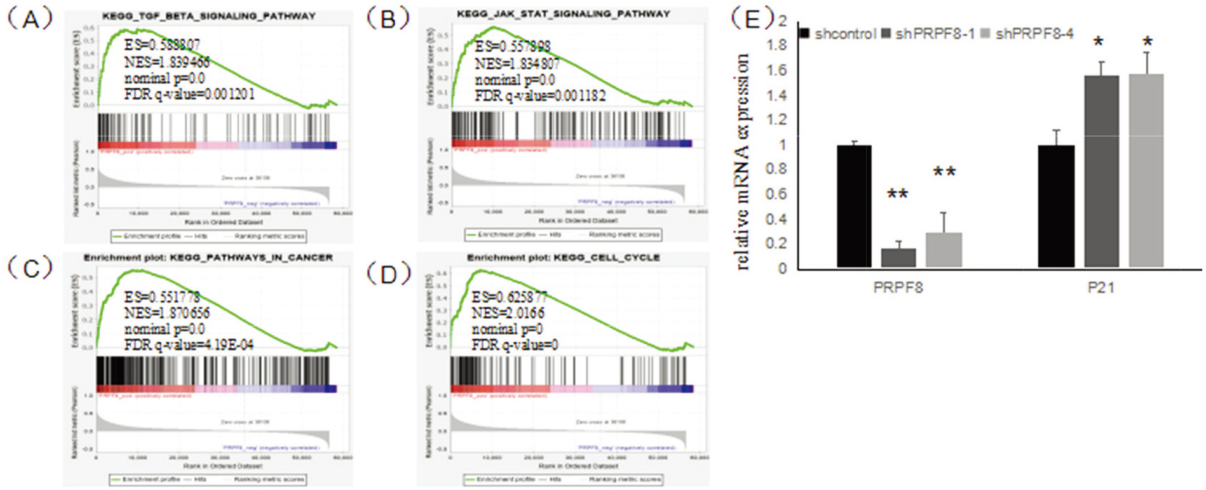

Fig. 4. (A) GSEA results show TGF-β signaling pathway is associated with *PRPF8* expression. (B) GSEA results show JAK-STAT pathway is associated with *PRPF8* expression. (C) GSEA results show pathways in cancer are associated with *PRPF8* expression. (D) GSEA results show cell cycle pathway is associated with *PRPF8* expression. (E) Inhibition of *PRPF8* expression upregulated $p21$ mRNA expression.

3.4. PRPF8 regulates the expression of p21 in breast cancer

To further explore the functional mechanism of *PRPF8* in the breast cancer cell, we used GSEA to analyze the pathways associated with *PRPF8* in breast cancer. The results have shown that *PRPF8* positively regulates 153 pathways and negatively regulated 31 pathways. The high expression of *PRPF8* was correlated with JAK-STAT signaling pathway (ES = 0.557898, $p = 0$, FDR = 0.001182), TGF-β signaling pathway (ES = 0.588807, $p = 0$, FDR = 0.001201), pathway in cancer (ES = 0.551778, $p = 0$, FDR = 4.19E-04), and cell cycle pathway (ES = 0.625877, $p = 0$, FDR = 0). As cell cycle pathway is a key pathway that regulates cell growth, we selected $p21$, a critical component of the cell cycle pathway, to verify the GSEA results. After *PRPF8* shRNAs were transduced into MCF-7 cells, the mRNA expression of $p21$ was detected with quantitative PCR (q-PCR). The result shown that inhibition of *PRPF8* expression up-regulated the expression of $p21$ in MCF-7cells (Fig. 4E). Therefore, *PRPF8* inhibition increased $p21$ expression in MCF-7 cells to inhibit the cell proliferation.

4. Discussion

In this study, we investigate the role of *PRPF8* in breast cancer. *PRPF8* mRNA expression is significantly elevated in breast cancer samples compared with the paracancerous tissue. *PRPF8* mRNA is differentially expressed among different breast cancer molecular subtypes, and its levels were inversely correlated with the OS in breast cancer patients. Furthermore, we confirmed the role of *PRPF8* in breast cancer with *in vitro* experiments, which have shown that silencing *PRPF8* in breast cancer cells repressed cell proliferation by upregulating $p21$ expression.

Alternative splicing is one of the mechanisms to increase protein diversity [5–9]. Recently, with the better understanding of alternative splicing process [10–15], it has been found that abnormal expression of splicing factors is closely related to many diseases. Many splicing factors play an important role in

cancer [16–23], including in breast cancer [24–26]. *PRPF8* is the core component of the ribonucleoprotein (RNP) complexes in the spliceosome and participates in splice-site recognition, branch-point formation and catalysis process [27–29]. Whether *PRPF8* plays a role in breast cancer is not known.

In this study, we demonstrate that *PRPF8* is critical for breast cancer cell survival. Firstly, *PRPF8* is elevated in breast tumors compared with the normal tissue (Fig. 1B). Second, *PRPF8* was related to OS, PPS, and DMFS in breast cancer patients (Fig. 2A, B&C). More importantly, silencing of *PRPF8* slowed down breast cancer cell growth and reduced the colony formation of MCF-7 cells (Fig. 3). Therefore, we found *PRPF8* plays an important role in breast cancer.

$p21$ is a cyclin-dependent kinase inhibitor [30–34], It binds to cyclin-dependent kinase 2 complexes and inhibits their activity [35,36]. Previous research has shown that co-expression of $p21$ and $p27$ proteins in MCF-7 cells induced cell apoptosis and inhibited cell proliferation [37]. Another study has shown that tumor growth was significantly reduced by transferring $p21$ into breast cancer mouse model cell lines by inhibiting cell proliferation [38]. Our results indicated that *PRPF8* expression was associated with many pathways, such as TGF-β pathway, JAK-STAT pathway, cell cycle control pathway. Recent studies have proved that multiple pathways are related to $p21$, including cell cycle, TGF-β, and JAK-STAT pathways [39–44]. Our study demonstrated that silencing *PRPF8* up-regulated the expression of $p21$ and inhibited cancer cell survival.

5. Conclusion

Our study provides evidence that splicing factor *PRPF8* is critical for breast cancer cell survival and has the potential prognostic value in breast cancer. *PRPF8* may achieve its functions in breast cancer by modulating $p21$ expression.

Conflict of interest

The authors report no conflict of interest.

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