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Original Article

HnRNP-F promotes cell proliferation by regulating TPX2 in bladder cancer

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Abstract: Heterogeneous nuclear ribonucleoprotein F (hnRNP-F) is crucial for gene expression and signal transduction as a tumor-promoting molecule with the ability to promote cell proliferation in various cancers. However, the role and mechanism of hnRNP-F in bladder cancer (BC) remain unclear. Therefore, we investigated the effect of hnRNP-F on the proliferation of BC cells and the potential mechanism. In this study, hnRNP-F was found to be upregulated in BC tissues and cells by western blotting. The knockdown of hnRNP-F could inhibit proliferation and delay cell cycle progression in EJ and UMUC-3 cells. Mechanistically, hnRNP-F was shown to bind to Targeting protein for Xenopus kinesin-like protein 2 (TPX2) by mass spectrometry and coimmunoprecipitation. Furthermore, Pearson correlation analysis showed that the expression of hnRNP-F was positively associated with that of TPX2 in BC tissues ($P<0.001$, $r=0.8180$). Notably, TPX2 was correspondingly markedly decreased in cells upon hnRNP-F knockdown. In addition, the decrease in TPX2 after hnRNP-F knockdown further decreased cyclin D1 protein expression and evoked p21 protein expression, eventually resulting in cell cycle arrest and proliferation inhibition in BC cells. Moreover, the overexpression of TPX2 protein was found to reverse the effect of hnRNP-F knockdown on the cell cycle and cell proliferation in BC cells. In conclusion, these findings suggest that hnRNP-F could promote cell proliferation and drive cell cycle progression by regulating TPX2 in BC, which may serve as a potential target for the treatment of BC patients.

Keywords: Heterogeneous nuclear ribonucleoprotein F, targeting protein for Xenopus kinesin-like protein 2, bladder cancer, cell proliferation

Introduction

Human bladder cancer (BC), one of the most common genitourinary malignancies worldwide, remains a major threat to public health. The incidence and mortality of BC have increased dramatically in recent decades [1]. Multigene, multifactor and multistep processes are involved in the carcinogenesis and development of BC, including aberrant cell proliferation [2]. The pathological mechanism of cancerous cell proliferation is a complex process initiated by the accumulation of genetic and epigenetic alterations and has recently received much attention [3, 4]. Therefore, it is of great importance to identify novel therapeutic targets for the prognostic prediction and treatment of BC.

Heterogeneous nuclear ribonucleoprotein F (hnRNP-F), belonging to the hnRNP family, plays important roles in gene expression and signal transduction, including pre-mRNA and mature mRNA transcription, alternative splicing and polyadenylation [5]. Aberrant expression of hnRNP-F has been observed in various cancers, including colon cancer, gastric cancer and prostate cancer [6]. HnRNP-F was reported to mediate the proliferation of Hek293 and A549 cells via mTOR and S6K2, which may be one of the mechanisms underlying the tumorigenicity of hnRNP-F [7]. However, the role and mechanism of hnRNP-F in BC proliferation remain poorly understood.

Targeting protein for Xenopus kinesin-like protein 2 (TPX2), a microtubule-related protein, accumulates at the poles of metaphase spindles and regulates the assembly of the mitotic spindle [8]. Marked aberrant expression of TPX2 was found in various tumors, including

BC, which promoted the proliferation, tumorigenicity and metastasis of tumor cells [9, 10]. Noticeably, TPX2 was involved in the cell cycle and predominantly expressed in proliferating cells from the G1/S transition [11, 12] and was demonstrated to regulate the expression of cyclin D1 and p21 to promote the proliferation of human BC T24 cells [10]. Nevertheless, the relationship between hnRNP-F and the TPX2 protein is unclear.

Here, we demonstrate that hnRNP-F expression is significantly increased in human BC tissues and cell lines and investigate the effect of aberrant hnRNP-F expression on BC cell proliferation. We present evidence that the interaction of hnRNP-F with the TPX2 protein regulates the cell cycle and proliferation of BC cells. Our findings reveal new insights into the function and mechanism of hnRNP-F in pathogenesis and provide a potential therapeutic target for BC.

Materials and methods

Antibodies

HnRNP-F rabbit polyclonal antibody was acquired from Novus Biologicals (USA), and TPX2 mouse monoclonal antibody was obtained from Abcam (UK). IgG rabbit polyclonal antibody, IgG mouse polyclonal antibody, Alexa Fluor 594-conjugated goat anti-rabbit IgG (H+L), Alexa Fluor 488-conjugated goat anti-mouse IgG (H+L), HRP-conjugated goat anti-rabbit IgG (H+L), HRP-conjugated goat anti-mouse IgG (H+L) antibodies and antibodies against KI67, p21, GAPDH and Cyclin D1 were obtained from Proteintech (China).

Clinical specimens

Ten BC specimens and paired adjacent tissues were obtained from newly diagnosed BC patients who underwent radical cystectomy at the Department of Urology, Nanfang Hospital (Guangzhou, China) between November 2014 and July 2017. The diagnosis was performed by two pathologists at Nanfang Hospital. All tissues were collected with informed consent from patients and were authorized by the Nanfang Hospital Institutional Board.

Cell culture

The human BC cell lines 5637, T24, EJ and UMUC-3 were cultured in RPMI-1640 (Gibco, USA) with 10% fetal bovine serum (Serana, Germany) and 1% penicillin/streptomycin (Solarbio, China) at 37°C in a humidified incubator with 5% CO_2 atmosphere.

Western blotting

Proteins from BC cells and tissues were separated by SDS-PAGE and then transferred onto PVDF membranes (Millipore, USA). After blocking, the membranes were incubated with different specific antibodies (hnRNP-F, 1:1000; TPX2, 1:1000; cyclin D1, 1:1000; p21, 1:1000; GAPDH, 1:5000), followed by incubation with HRP-conjugated secondary antibodies (1:5000). Immunoreactivity was visualized by ECL chemiluminescence (FDbio Science, China). The protein levels were normalized to GAPDH.

Stable knockdown of hnRNP-F

To stably repress the expression of hnRNP-F in BC cells, a short-hairpin RNA (shRNA) lentivirus targeting hnRNP-F was constructed (Geneparma, China) with a sequence of GGUACAUUGAGGUGUUCAATT. After transfection, cells with shRNA lentivirus (sh-hnRNP-F) or negative control virus (con-hnRNP-F) were screened using 5 μg/ml puromycin (Sigma, USA). Transfection efficiency was monitored by western blotting.

MTT assay

A total of 2×10^3 cells per well were placed in 96-well plates. Then, 10 μl MTT reagent (5 mg/ml; solarbio, China) was added to each well and incubated for 4 h. After the removal of the MTT solution, 100 μl dimethyl sulfoxide (DMSO; Solarbio, China) was added to dissolve formazan crystals. The absorbance at 490 nm was determined.

Colony formation assay

A total of 400 cells per well were added to 6-well plates and cultured for 10 days. The clones were fixed with paraformaldehyde, followed by staining with Giemsa (Solarbio, China). The clones were photographed and counted.

Cell cycle analysis

The cell cycle of BC cells was examined with a flow cytometry assay. Cells in log-phase were harvested and fixed in 70% ice-cold alcohol. The cell pellets were stained with 100 μg/ml

RNAse and 50 μg/ml propidium iodide (PI), incubated for 30 min, and then analyzed by flow cytometry (FACSCalibur Becton Dickinson).

Immunocytochemistry

The KI67 protein was examined by an immunohistochemical detection kit (Zhongshan Biotech, China) according to the manufacturer's instructions. Briefly, cells were cultured and fixed with paraformaldehyde on cover slides in 6-well plates. After that, the cells were incubated with an endogenous peroxidase blocker, KI67 antibody (1:1000) and HRP-conjugated goat anti-rabbit IgG polymer and then stained with DAB.

The results were independently scored by two observers. The staining scoring criteria were as follows: no color, 0 points; light yellow, 1 point; yellow granules, 2 points; brown granules, 3 points. The percentage of positive cells was scored as follows: <5%, 0 points; 6-25%, 1 point; 26-50%, 2 points; 51-75%, 3 points; ≥76%, 4 points [13]. These two scores were multiplied to obtain the final scores, which were used for statistical analysis.

Mass spectrometry (MS) and coimmunoprecipitation (Co-IP)

Cell pellets were treated with RIPA lysis buffer (FDbio Science, China) and followed by centrifugation. Then, the supernatant was separated into three centrifugal tubes (input, positive control; IgG, negative control; hnRNP-F, treatment). The input protein sample was added with only 25 μl 5×SDS-PAGE Sample Loading Buffer (FDbio Science, China). The IgG and hnRNP-F groups were preincubated with 20 μl magnetic beads. Then, the supernatant separated by a magnetic separator was incubated with 5 μl specific primary antibody and 40 μl magnetic beads. Precipitation complex-bound beads were washed and resuspended in 50 μl 1×SDS-PAGE Sample Loading Buffer. All protein samples were boiled and separated by gel electrophoresis. After sliver staining, the gel was analyzed by MS (Wininnovate Bio, China). For Co-IP, degenerated protein samples were tested by western blotting.

Immunofluorescence analysis

Cells were cultured and fixed on confocal dishes. After blocking, the dishes were incubated with hnRNP-F antibody (1:100) and TPX2 antibody (1:100) and then with a mixture of Alexa Fluor 594-conjugated goat anti-rabbit IgG (H+L) and Alexa Fluor 488-conjugated goat anti-mouse IgG (H+L) (1:150). Then, the cells were mounted with DAPI (1:100; Proteintech, China). The images were photographed with inverted laser confocal microscopy (Carl Zeiss, Germany), and the fluorescence values were calculated by the associated Carl Zeiss confocal microscopy viewer (Carl Zeiss, Germany).

Construction of plasmid and transfection

To establish BC cell lines with hnRNP-F knockdown or overexpression, the full-length TPX2 sequence was subcloned into a pENTER vector (TPX2 OE) and an empty pENTER vector (conTPX2 OE) was used as a control, while TPX2 shRNA (shTPX2) and nontargeting shRNA (conshTPX2) were generated using pLent-U6-GFP-Puro vectors (Vigene Bioscience, China). The shRNA target sequence was 5'-AAGAATGGAACTGGAGGGCTT-3'. The transfection assay was performed using Hieff Trans Liposomal Transfection Reagent (Yeasen, China) following the instructions.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 software (SPSS Inc., USA). An independent samples T-test was used to analyze continuous data. A paired-samples t-test was used to compare the levels of hnRNP-F and TPX2 between BC and paired adjacent tissues. Linear regression was used to determine the correlation between hnRNP-F and TPX2 proteins. A p value less than 0.05 was considered statistically significant. All experiments were conducted in triplicate.

Results

HnRNP-F was increased in human BC tissues and cells

The expression levels of hnRNP-F were detected by western blotting in a panel of BC tissues and cell lines. An increase in hnRNP-F expression was observed in BC tissues compared with paired adjacent control tissues (P<0.01, **Figure 1A**). Furthermore, the levels of hnRNP-F were significantly higher in EJ and UMUC-3 cells than in 5637 and T24 cells (P<0.001, **Figure 1B**). Therefore, EJ and UMUC-3 cells were used for further analysis.

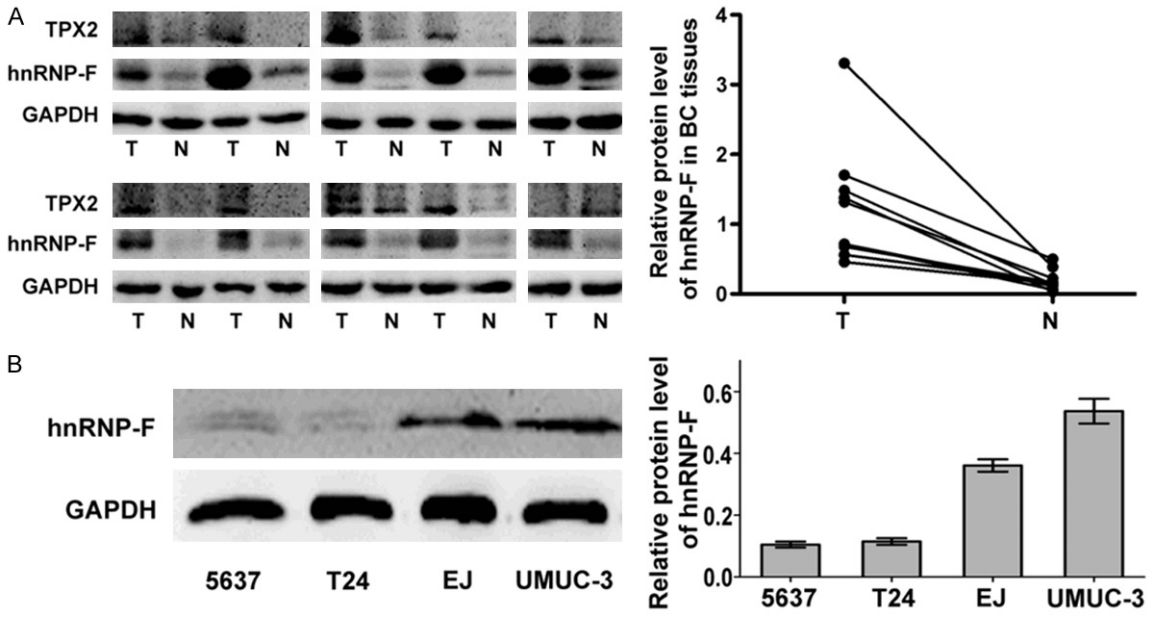


Figure 1. HnRNP-F and TPX2 were upregulated in BC. A. HnRNP-F and TPX2 proteins were detected in ten cases of BC (T) and adjacent noncancer tissues (N). B. HnRNP-F protein was detected in the 5637, T24, EJ and UMUC-3 cell lines.

HnRNP-F could promote BC cell proliferation and accelerate cell cycle progression

To demonstrate the role of hnRNP-F in BC cell proliferation, a shRNA lentivirus targeting hnRNP-F was transfected into EJ and UMUC-3 cells to inhibit endogenous hnRNP-F expression. Western blotting analyses showed that the hnRNP-F protein level was markedly reduced in sh-hnRNP-F cells compared with the control or con-hnRNP-F cells (EJ, $P<0.05$ and UMUC-3, $P<0.001$, **Figure 2A**). An MTT assay was conducted to examine the biological effect of hnRNP-F on cell proliferation, and the results indicated that the depletion of hnRNP-F notably inhibited the growth of BC cells *in vitro* (EJ, $P<0.001$ and UMUC-3, $P<0.001$, **Figure 2B**). Similarly, the capacity to form colonies was suppressed in the hnRNP-F-depleted group compared with the control group (EJ, $P<0.01$ and UMUC-3, $P<0.001$, **Figure 2C**). In addition, according to the immunocytochemical analysis of the nuclear expression of Ki67, a cell proliferation marker [14], KI67 was decreased in the BC cell lines EJ (n=12, $P<0.001$) and UMUC-3 (n=12, $P<0.05$) with hnRNP-F depletion compared with control groups (**Figure 2D**), indicating that the proliferation of BC cells was decreased upon hnRNP-F knockdown. Briefly, these data indicate that hnRNP-F is necessary for the cell proliferation of BC.

Abbreviated cell cycle progression plays an important role in aberrant cell proliferation [10]. The effect of hnRNP-F on cell proliferation impelled us to further explore the role of hnRNP-F in cell cycle progression. The results from the flow cytometry assay showed that the percentages of G1-phase cells were increased in the EJ ($P<0.001$) and UMUC-3 ($P<0.001$) cell lines with hnRNP-F knockdown compared with the con-hnRNP-F groups, while the percentages of S-phase cells (EJ, $P<0.001$ and UMUC-3, $P<0.001$) were decreased (**Figure 2E**). These data indicated that hnRNP-F could promote the G1/S transition of BC cells, which may be one of the mechanisms by which hnRNP-F promotes cell proliferation in BC.

HnRNP-F bound to and was positively associated with TPX2

To search for putative proteins related to hnRNP-F involved in cell cycle regulation, the MS analysis of hnRNP-F immunoprecipitation from EJ cells identified many proteins, including TPX2 (**Figure 3A**). Interestingly, previous studies have demonstrated that TPX2 plays key roles in the cell cycle and in proliferating cells

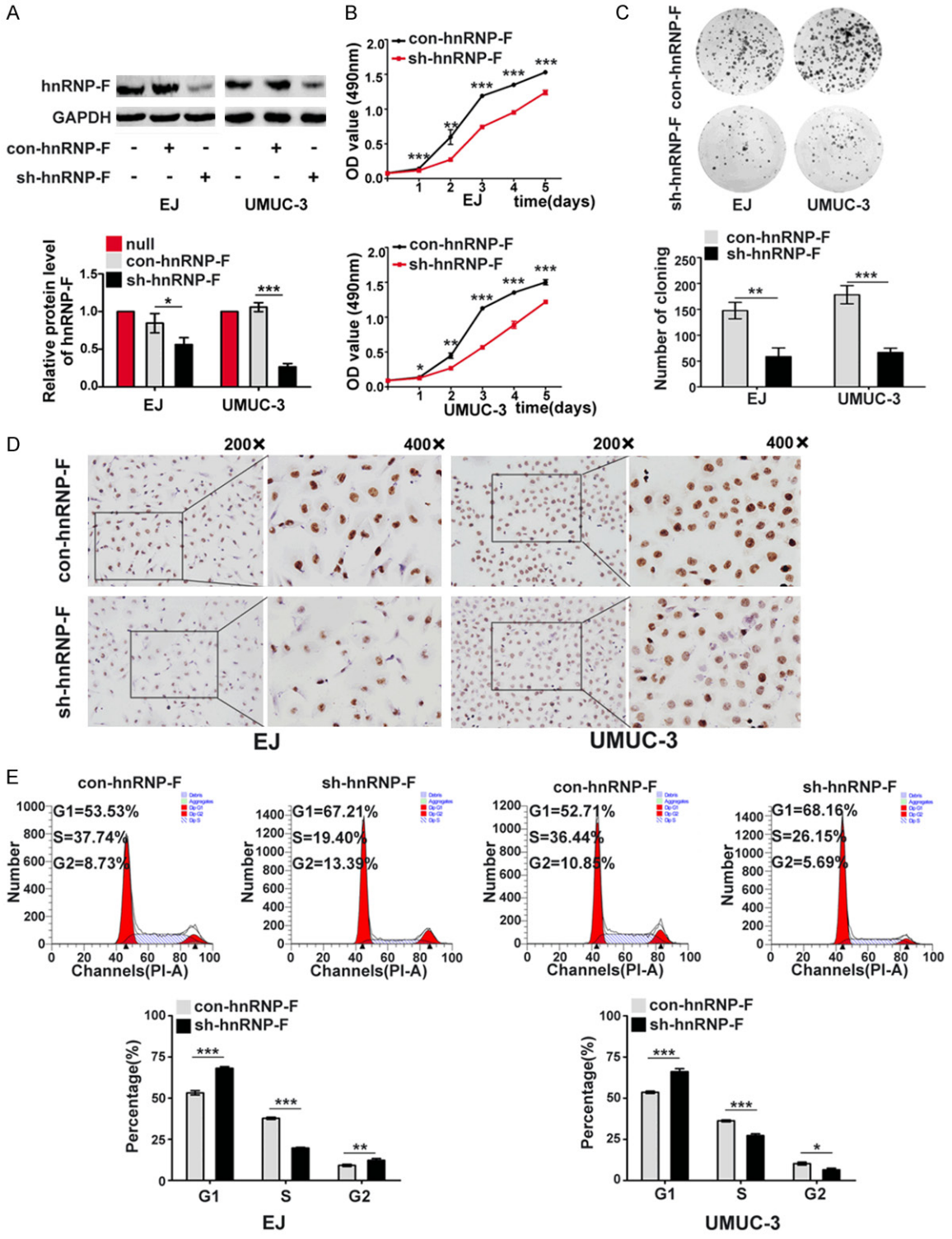


Figure 2. Knockdown of hnRNP-F could inhibit cell proliferation and cell cycle progression in BC cells *in vitro* (*P<0.05, **P<0.01 and ***P<0.001). (A) The transfection of sh-hnRNP-F was performed to establish EJ and UMUC-3 cells with the stable knockdown of hnRNP-F expression. HnRNP-F levels were detected in EJ and UMUC-3 cells after hnRNP-F knockdown by western blotting. MTT assay (B) and colony formation assay (C) were performed to detect the effect of hnRNP-F knockdown on the cell proliferation of EJ and UMUC-3 cells. (D) Immunocytochemistry analysis of KI67 protein was performed in EJ and UMUC-3 cells. (E) Cell cycle distribution of EJ and UMUC-3 cells was analyzed by flow cytometry.

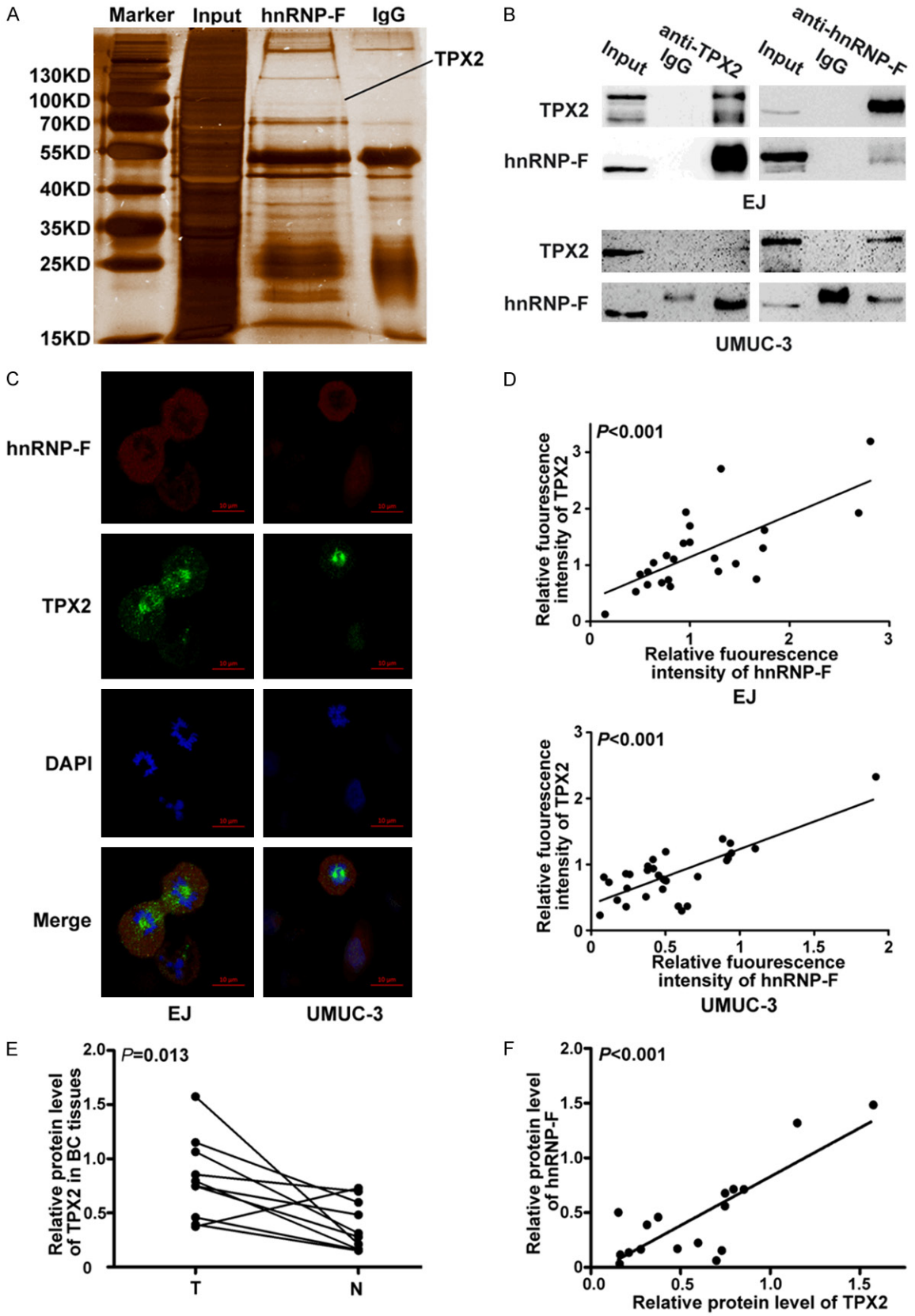


Figure 3. HnRNP-F bound to and was positively associated with TPX2. A. TPX2 was associated with hnRNP-F as determined by immunoprecipitation and mass spectrometry. B. The relationship between hnRNP-F and TPX2 protein

was detected by a series of coimmunoprecipitation assays in EJ and UMUC-3 cells. C. Immunofluorescent staining was performed to investigate the expression of hnRNP-F (red) and TPX2 (green). D. Pearson correlation analyses were performed to determine the correlation between hnRNP-F and TPX2. E. The expression levels of TPX2 in human BC tissues (T) and adjacent noncancer tissues (N) were calculated using Paired-Samples T-test. F. The association of hnRNP-F with TPX2 in human BC tissues was analyzed.

[11, 12]. Furthermore, a Co-IP assay was performed to validate the interaction between hnRNP-F and TPX2. The hnRNP-F protein was visualized with an anti-TPX2 antibody in both EJ and UMUC-3 cells, while the TPX2 protein was also visualized with an anti-hnRNP-F antibody (**Figure 3B**). The data indicated that hnRNP-F physically bound to the TPX2 protein in the EJ and UMUC-3 cell lines.

Immunofluorescence analysis was used to explore the expression levels and distribution of hnRNP-F and TPX2 proteins in EJ and UMUC-3 cells. Unsurprisingly, hnRNP-F (red) and TPX2 (green) were observed in both the cytoplasm and nucleus, supporting hnRNP-F binding to TPX2 (**Figure 3C**). Relative fluorescence density value analyses demonstrated that hnRNP-F was positively associated with TPX2 in EJ ($P<0.001$) and UMUC-3 ($P<0.001$) cells (**Figure 3D**), and the Pearson correlation coefficients were 0.7038 and 0.7687, respectively.

It has been reported that TPX2 expression is increased in multiple tumors [9, 10]. Our results revealed that an increase in TPX2 was found in BC tissues compared with paired adjacent tissues (9/10; $P<0.05$, **Figures 1A**, **3E**). Pearson correlation analysis showed that the expression of hnRNP-F was positively associated with that of TPX2 in BC tissues ($P<0.001$, r=0.8180, **Figures 1A**, **3F**).

HnRNP-F regulated cyclin D1 and p21 through TPX2

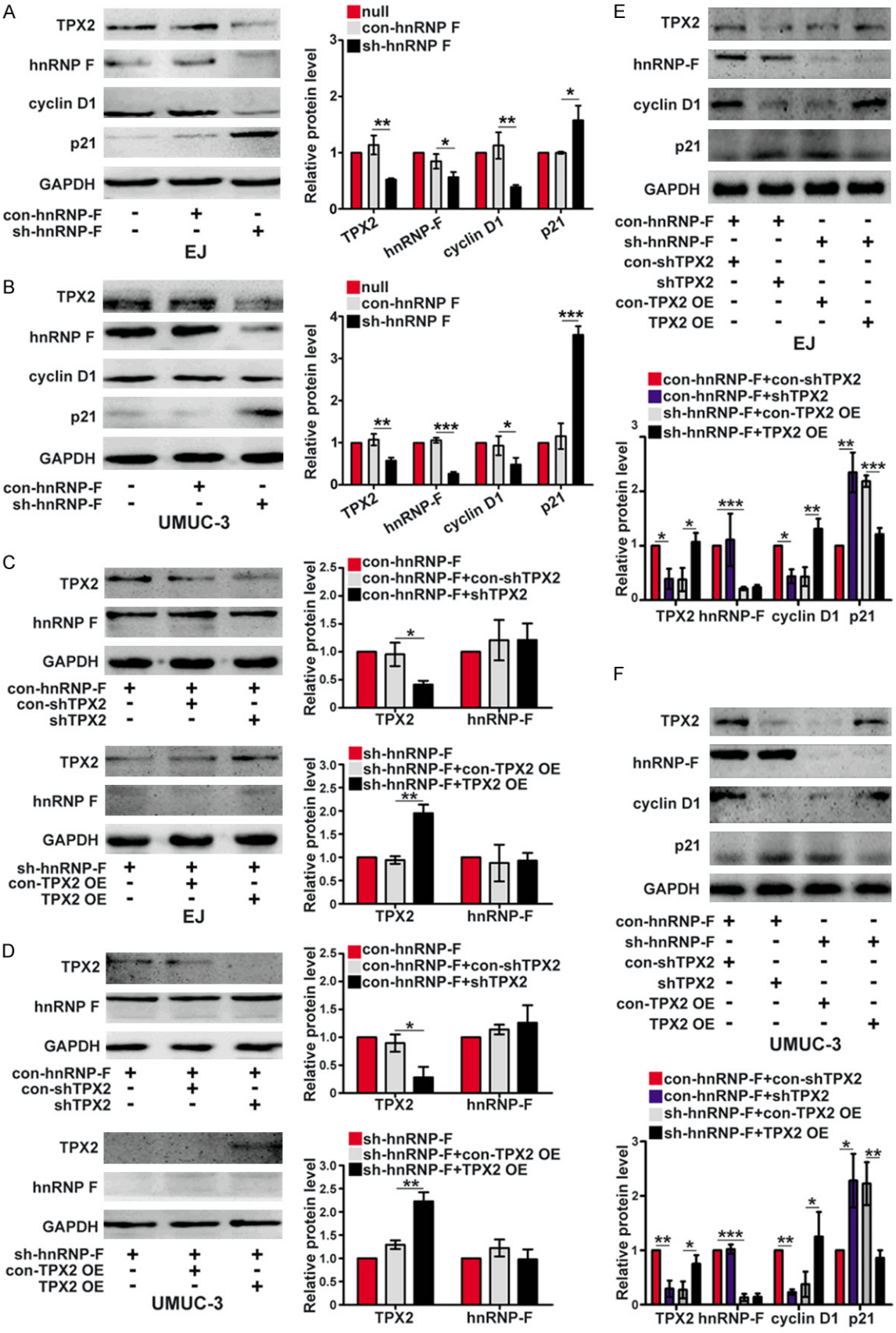
HnRNP-F could accelerate the cell cycle progression of EJ and UMUC-3 cells. Western blotting analysis was performed to investigate the effect of hnRNP-F on the expression of the cell cycle-related proteins cyclin D1 and p21 and showed that the cyclin D1 protein was decreased in EJ ($P<0.01$) and UMUC-3 ($P<0.05$) cells with hnRNP-F knockdown compared with the control cells, whereas the p21 protein was increased (EJ, $P<0.05$ and UMUC-3, $P<0.001$, **Figure 4A**, **4B**). The alterations in cyclin D1 and p21 could be responsible for the delay of cell cycle progression induced by hnRNP-F knockdown. Notably, TPX2 was correspondingly markedly decreased (EJ, $P<0.01$ and UMUC-3, $P<0.01$) in cells upon hnRNP-F knockdown. Because TPX2 protein could alter cyclin D1 and p21, we speculated that hnRNP-F might regulate the expression of cyclin D1 and p21 in BC cells through TPX2.

TPX2 is required for hnRNP-F to promote cell proliferation and cell cycle distribution

To investigate the role of TPX2 protein in the hnRNP-F-mediated promotion of cell proliferation and cell cycle progression, we then sought to transfect TPX2 shRNA or the pENTER-TPX2 vector into cells with con-hnRNP-F or sh-hnRNP-F, respectively. The results from EJ cells showed that the level of TPX2 in the con-hnRNP-F+shTPX2 group was significantly lower than that in the con-hnRNP-F+con-shTPX2 group ($P<0.05$), and a higher TPX2 expression was observed in the shRNP-F+TPX2 OE group than in the shRNP-F+con-TPX2 OE group ($P<0.01$, **Figure 4C**). A similar result was observed in UMUC-3 cells (**Figure 4D**). Interestingly, the expression of hnRNP-F was not significantly changed upon TPX2 knockdown or overexpression in either EJ or UMUC-3 cells, indicating that TPX2 is a downstream target of hnRNP-F.

To further determine whether the expression levels of cyclin D1 and p21 were mediated by hnRNP-F through TPX2, the relationships between TPX2 and cyclin D1 or p21 were detected by western blotting. Decreased cyclin D1 and increased p21 were observed in EJ and UMUC-3 cells with con-hnRNP-F+shTPX2 compared with con-hnRNP-F+con-shTPX2 cells (**Figure 4E**, **4F**). Meanwhile, increased cyclin D1 and decreased p21 were detected in sh-hnRNP-F+TPX2 OE cells versus sh-hnRNP-F+con-TPX2 OE cells (**Figure 4E**, **4F**).

The role of TPX2 in the proliferation and cell cycle progression of BC cells mediated by hnRNP-F was investigated by MTT, colony formation and flow cytometry assays. The proliferation ability of the con-hnRNP-F+shTPX2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UMUC-3
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sh-hnRNP-F+con-TPX2 OE
sh-hnRNP-F+TPX2 OE
Relative protein level
TPX2 hnRNP-F cyclin D1 p21

Figure 4. HnRNP-F regulated cyclin D1 and p21 through TPX2 (*P<0.05, **P<0.01 and ***P<0.001). A, B. The protein expression of TPX2, cyclin D1 and p21 was detected by western blotting in EJ and UMUC-3 cells with or without hnRNP-F knockdown. C, D. TPX2 and hnRNP-F proteins were detected by western blotting in EJ and UMUC-3 cells with shTPX2 or TPX2 OE. E, F. Cyclin D1 and p21 proteins were detected by western blotting in EJ and UMUC-3 cells with shTPX2 or TPX2 OE.

groups was significantly decreased compared with the con-hnRNP-F+con-shTPX2 groups in EJ (P<0.001) and UMUC-3 (P<0.001) cells. In contrast, the proliferation ability of the sh-hnRNP-F+TPX2 OE groups was more vigorous than that of the sh-hnRNP-F+con-TPX2 OE groups in EJ (P<0.001) and UMUC-3 (P<0.001) cells (**Figure 5A**). The results from the colony formation assays were consistent with those from the MTT assays (**Figure 5B**). Furthermore, the flow cytometry results indicated that compared with the control condition, the depletion of TPX2 resulted in upregulated G1-phase cell population and downregulated S-phase cell population, while the overexpression of TPX2 caused decreased G1-phase cell population and increased S-phase cell population in in both EJ and UMUC-3 cells (**Figure 5C**). Briefly, TPX2 is required for the hnRNP-F-mediated promotion of cell proliferation and cell cycle progression in BC.

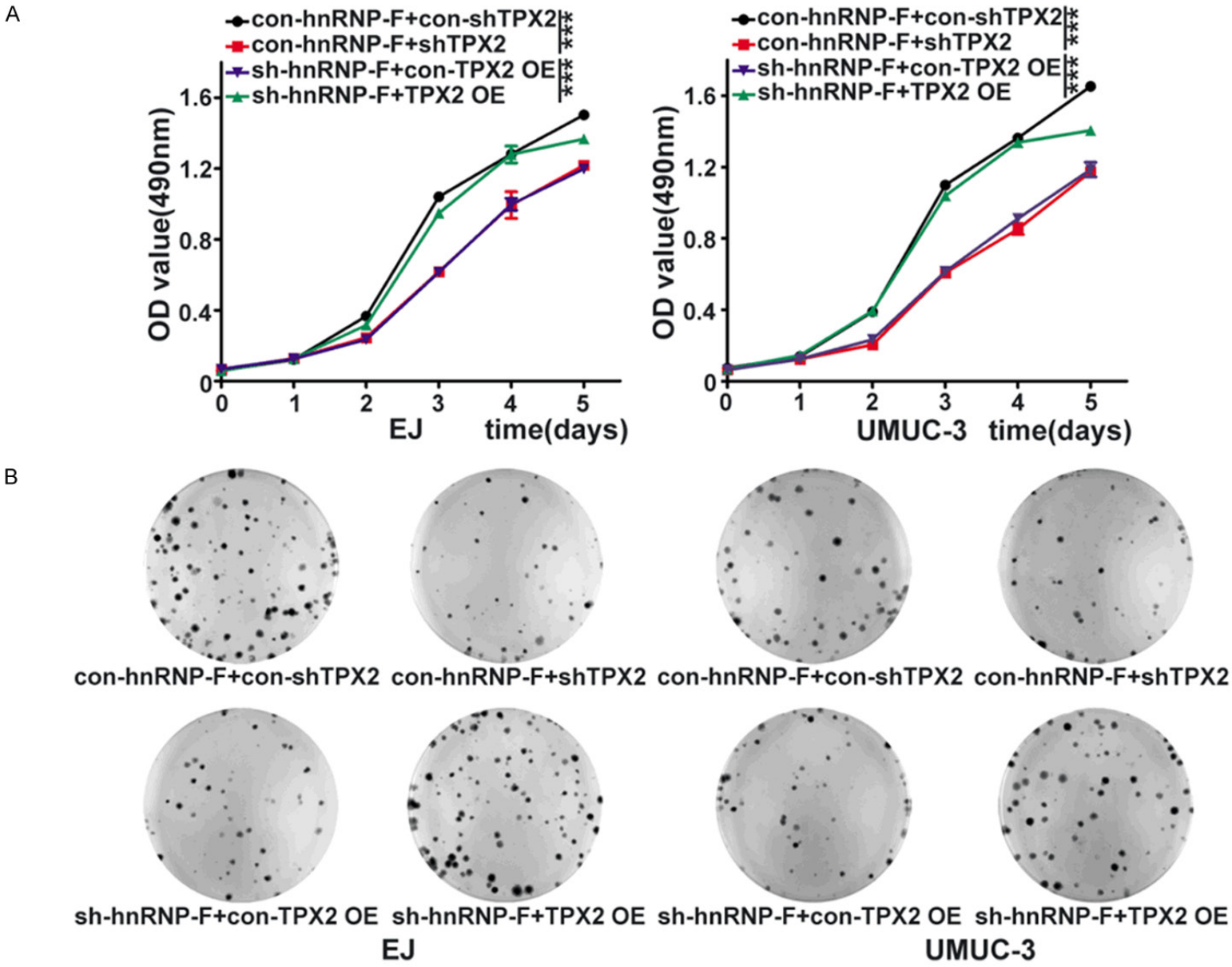
Discussion

BC is regarded as one of the most serious cancers. It is clinically characterized by high recurrent rates and a higher mortality rate once tumors progress to muscularis propria invasive disease. Although the treatment of BC has made great achievement in recent decades, the poor prognosis of patients with invasive stage tumor or metastatic disease remains a challenge [15]. The progression of BC has been demonstrated to be associated with proliferation and invasion [16]. Research on the mechanism of cancerous cell proliferation has received much attention recently because it is necessary to investigate the fundamental mechanisms of BC development to identify effective therapeutic targets to improve prognosis [17].

The heterogeneous nuclear ribonucleoprotein (hnRNPs) has been demonstrated to assist in controlling pre-mRNA and mature mRNA transcription, alternative splicing, polyadenylation, cellular signal transduction, translation and degradation [18]. Emerging evidence has revealed that the aberrant expression of hnRNPs contributes to the progression of cancer. For example, hnRNP A2/B1 protein was aberrantly overexpressed in human hepatocellular carcinoma (HCC), and the alteration of its localization indicated poor prognosis during the development of HCC [19]. HnRNP A/B was also significantly increased in non-snonsmall cell lung cancer, and its increased expression level was tightly associated with poor prognosis [20]. HnRNP K was proven to be upregulated in BC by promoting cell proliferation, antiapoptosis effects and chemoresistance to cisplatin by regulating a series of genes at the transcriptional level [21].

HnRNP-F, a member of the hnRNP family, is well known for its function in the regulation of alternative splicing [22]. Moreover, many previous studies have reported that show hnRNP-F expression is altered in many types of cancer, suggesting its roles in tumorigenesis. Honore, B. and coworkers [6] observed that the expression level of hnRNP-F was enhanced in gastric carcinoma, and Xu SH [23] found that Drosophila ecdysoneless gene (ECD) facilitated gastric cancer migration and invasion by stabilizing hnRNP-F. However, the association of hnRNP-F with BC is unclear.

In our study, we studied a cohort of BC patients and determined that hnRNP-F expression is significantly increased in human BC tissues compared with paired adjacent noncancer tissues. Meanwhile, knockdown of hnRNP-F could inhibit the proliferation of EJ and UMUC-3 cells *in vitro,* which was further confirmed by the KI67 index with immunocytochemistry. We further found that hnRNP-F knockdown could inhibit the G1/S transition, indicating that the ability to mediate the cell cycle may be one of the mechanisms of hnRNP-F in promoting cell proliferation. Briefly, we demonstrated that hnRNP-F was upregulated in BC and promoted cell proliferation and accelerated cell cycle progression in BC, but the molecular mechanisms of hnRNP-F targeting in BC and, particularly, the effects on cell proliferation and cycle progression remain unclear.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1.6
0
1
2
3
4
5
UMUC-3
time(days)
B
con-hnRNP-F+con-shTPX2
con-hnRNP-F+shTPX2
con-hnRNP-F+con-shTPX2
con-hnRNP-F+shTPX2
sh-hnRNP-F+con-TPX2 OE
sh-hnRNP-F+TPX2 OE
sh-hnRNP-F+con-TPX2 OE
sh-hnRNP-F+TPX2 OE
EJ
UMUC-3

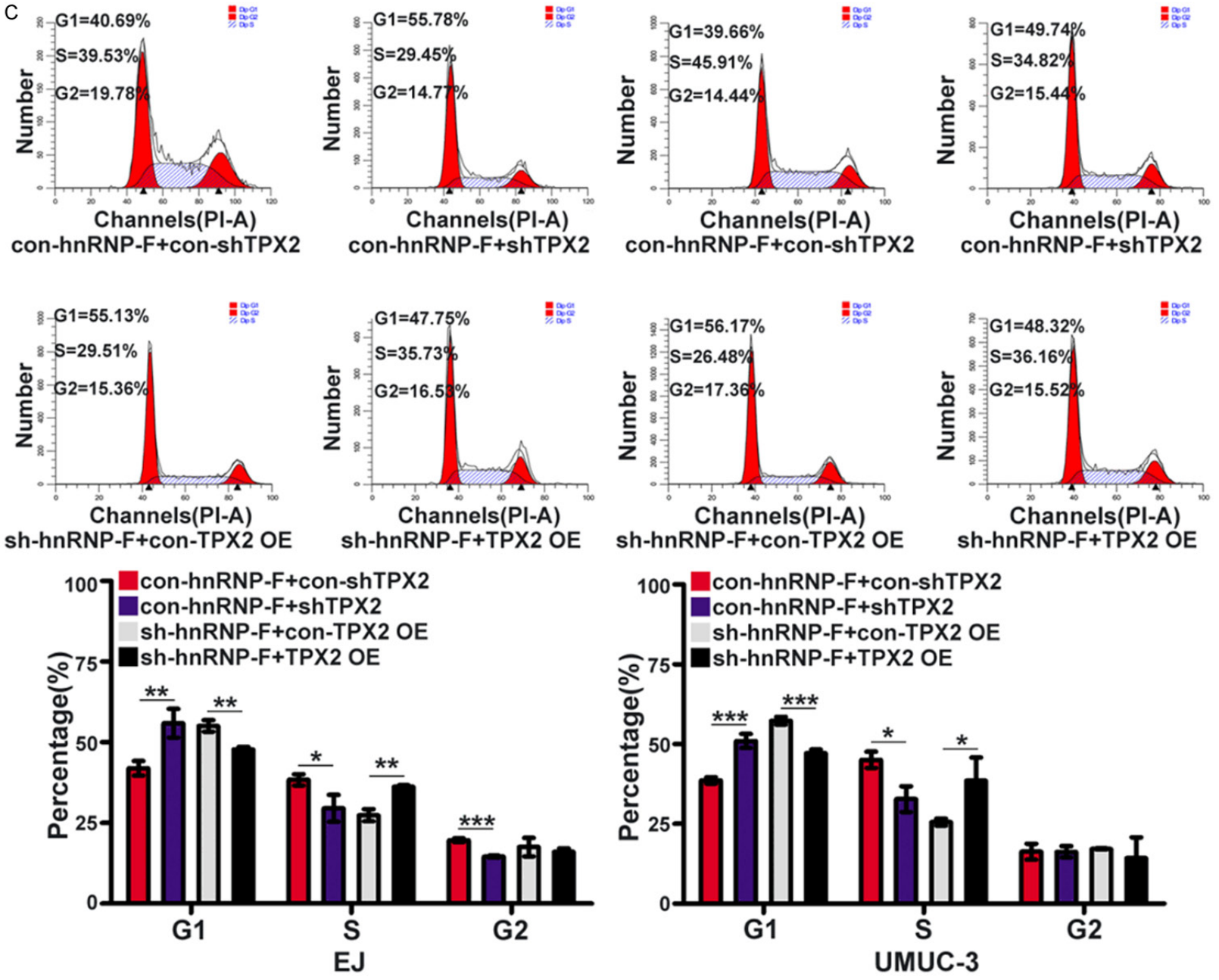
C
G1=40.69%
S=39.53%
G2=19.78%
Number
Channels(PI-A)
con-hnRNP-F+con-shTPX2
G1=55.78%
S=29.45%
G2=14.77%
Number
Channels(PI-A)
con-hnRNP-F+shTPX2
G1=39.66%
S=45.91%
G2=14.44%
Number
Channels(PI-A)
con-hnRNP-F+con-shTPX2
G1=49.74%
S=34.82%
G2=15.44%
Number
Channels(PI-A)
con-hnRNP-F+shTPX2
G1=55.13%
S=29.51%
G2=15.36%
Number
Channels(PI-A)
sh-hnRNP-F+con-TPX2 OE
G1=47.75%
S=35.73%
G2=16.53%
Number
Channels(PI-A)
sh-hnRNP-F+TPX2 OE
G1=56.17%
S=26.48%
G2=17.36%
Number
Channels(PI-A)
sh-hnRNP-F+con-TPX2 OE
G1=48.32%
S=36.16%
G2=15.52%
Number
Channels(PI-A)
sh-hnRNP-F+TPX2 OE
con-hnRNP-F+con-shTPX2
con-hnRNP-F+shTPX2
sh-hnRNP-F+con-TPX2 OE
sh-hnRNP-F+TPX2 OE
Percentage(%)
100
75
50
25
0
**
**
*
**
***
G1
S
G2
EJ
con-hnRNP-F+con-shTPX2
con-hnRNP-F+shTPX2
sh-hnRNP-F+con-TPX2 OE
sh-hnRNP-F+TPX2 OE
Percentage(%)
100
75
50
25
0
***
***
*
*
G1
S
G2
UMUC-3

Figure 5. TPX2 could rescue the alterations in cell proliferation and cell cycle distribution caused by hnRNP-F knockdown (*P<0.05, **P<0.01 and ***P<0.001). MTT assay (A) and colony formation assay (B) were performed to detect the role of TPX2 on the cell proliferation of EJ and UMUC-3 cells mediated by hnRNP-F. (C) A flow cytometry assay was conducted to examine the role of TPX2 in the cell cycle distribution of EJ and UMUC-3 cells mediated by hnRNP-F.

TPX2, a 100 kDa protein, was first reported in 1997 by Heidebrecht et al. detecting the expression of which was induced from the G1/S transition to cytokinesis [24]. TPX2 was found to accumulate at the poles of metaphase spindles and promote the assembly of spindles [8]. TPX2 has been shown to play critical roles in the chromosome segregation machinery during mitosis, and its enhanced expression in prostate cancer could promote cancer cell growth, reduce apoptosis, and increase tumorigenesis [25]. However, no study on the association of hnRNP-F with TPX2 has been reported. In the present study, we found that hnRNP-F could bind to and positively interact with TPX2 and that both of them were distributed in the nucleus and cytoplasm of EJ and UMUC-3 cells. Moreover, we observed a positive correlation between hnRNP-F and TPX2, as revealed by immunofluorescence values and western blot analysis based on tissue proteins. These results illustrated that TPX2 may be a potential mediator for hnRNP-F in regulating cell growth.

Several signaling pathways were demonstrated to be involved in cell proliferation, including the MAPK/Erk [26, 27], Wnt/β-catenin [28] and AKT/mTOR [29] signaling pathways. The discoveries of these pathways contributed to our understanding of tumorigenesis and the progression of cancer. Proverbially, the signaling pathways that cyclin D1 and p21 participate in are multitudinous, such as the PI3K/Akt and p53 signaling pathways. Yan L and coworkers [10] showed that the increased TPX2 in BC promoted cancerous cell proliferation and evoked an increase in cyclin D1 expression and a decrease in p21 expression. Some previous studies reported that both cyclin D1 and p21 were involved in cell cycle progression [30, 31]. The regulations of cyclin D1 and p21 by TPX2 protein may be one of the mechanisms through which TPX2 promotes cell proliferation. In this study, we found that the levels of cyclin D1 and TPX2 were decreased and that the levels of p21 were increased upon hnRNP-F knockdown in EJ and UMUC-3 cells.

However, no alteration was found in hnRNP-F expression in the TPX2-depleted cells, although the levels of cyclin D1 and p21 were changed. Unsurprisingly, reverse results were found in sh-hnRNP-F cells overexpressing TPX2. In addition, exogenous overexpression of TPX2 in EJ and UMUC-3 cells rescued the delayed cell cycle and arrested cell proliferation caused by hnRNP-F knockdown. Therefore, on the basis of our results, we detected that TPX2 was required for hnRNP-F to improve cell proliferation, accelerate cell cycle progression and affect alterations in cyclin D1 and p21 in BC, which may be a novel discovery of hnRNP-F in promoting the progression of BC.

In summary, our findings suggest that hnRNP-F is significantly upregulated in human BC tissues and cells. Furthermore, hnRNP-F could promote cell proliferation and drive cell cycle progression through binding to TPX2 in BC, which may serve as a potential target for the treatment of BC patients.

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Disclosure of conflict of interest

None.

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