

Effects of UPF1 expression on EMT process by targeting E-cadherin, N-cadherin, Vimentin and Twist in a hepatocellular carcinoma cell line

YAWEI LI^{1*}, TIANTIAN ZHANG^{1*}, SHUKUI QIN², RUI WANG¹, YUMEI LI¹,
ZHENG GUANG ZHOU¹, YUFO CHEN¹, QIONG WU¹ and FANG SU¹

¹Department of Medical Oncology, The First Affiliated Hospital of Bengbu Medical College, Bengbu, Anhui 233004;

²Department of Medical Oncology, PLA Cancer Center, Nanjing Bayi Hospital, Nanjing, Jiangsu 210002, P.R. China

Received February 24, 2018; Accepted July 30, 2018

DOI: 10.3892/mmr.2019.9838

Abstract. Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. It has been reported that HCC has a poor prognosis. In the majority of cases, once metastatic, HCC is incurable. To identify an effective treatment for HCC, it is important to understand the underlying molecular mechanisms of HCC-associated occurrence, proliferation, metastasis and carcinogenesis. In the present study, the role of Up-frameshift 1 (UPF1), a potential tumor suppressor, was investigated in the HCC cell lines. The expression levels of UPF1 in an HCC cell line were examined by reverse transcription-quantitative polymerase chain reaction. The expression levels of 19 key proteins in numerous signaling pathways were detected via protein array analysis in the presence of UPF1 overexpression. The present study further investigated the effects of UPF1 expression levels on the epithelial-mesenchymal transition (EMT) process by targeting E-cadherin, N-cadherin, Vimentin and Twist-related protein 1 (Twist). The results of the present study revealed that UPF1 was significantly downregulated in an HCC cell line. The majority of the proteins exhibited upregulated expression levels in the presence of UPF1 overexpression in the HCC cell line, Huh-7. Key proteins, including cluster of differentiation (CD)31 (platelet endothelial cell adhesion molecule-1), Vimentin, CD44, PCNA, Ki-67, N-Cadherin, Survivin, P53, Met and retinoblastoma exhibited a significant association with UPF1. Furthermore, western blotting indicated that the expression levels of N-cadherin, Vimentin and Twist were

notably upregulated while UPF1 was overexpressed; however, E-cadherin was downregulated and opposing observations were reported with protein array analysis. In summary, E-cadherin expression levels were regulated by the manifold, and UPF1, a potential tumor suppressor, may promote the EMT process in Huh-7 HCC cells. The findings of the present study suggested that UPF1 expression levels affected the EMT process by targeting E-cadherin, N-cadherin, Vimentin and Twist.

Introduction

Hepatocellular carcinoma (HCC) is a primary liver cancer with high mortality (1,2) and is one of the most common malignancies worldwide, particularly in Asia, Africa and Southern Europe (3). Numerous advanced methods and techniques have been recently developed and applied for the treatment of cancer, including chemotherapy, targeted therapy and immunotherapy from which therapeutic outcomes have been observed; however, the majority of HCC cases remain incurable once metastatic and poor prognosis is exhibited (4). To develop an effective treatment against HCC, understanding the underlying molecular mechanisms of the occurrence, proliferation, metastasis and carcinogenesis associated with HCC is required.

Epithelial-mesenchymal transition (EMT) is a biological process in which epithelial cells transform and exhibit a mesenchymal phenotype via a specific program (5). The EMT process serves an essential role in embryonic development, chronic inflammation, tissue reconstruction, cancer metastasis and a variety of fibrotic diseases; EMT also possesses unique characteristics of gene regulation, such as that for the downregulation of E-cadherin (6). In addition, it has been demonstrated that EMT exhibits a significant effect on malignant tumors, whereby epithelial cells transform to obtain migration and invasion abilities associated with malignancy (7). Thus, it is important to investigate the molecular mechanism underlying the EMT process and understand its pathological significance in tumor occurrence, development and metastasis.

A previous study demonstrated that Up-frameshift 1 (UPF1), a potential tumor suppressor, not only serves a key role in RNA degradation pathways, but exhibits significant effects

Correspondence to: Dr Fang Su or Dr Qiong Wu, Department of Medical Oncology, The First Affiliated Hospital of Bengbu Medical College, 287 Changhuai Road, Bengbu, Anhui 233004, P.R. China
E-mail: sufang2899@163.com
E-mail: qiongwu68@aliyun.com

*Contributed equally

Key words: Up-frameshift 1, hepatocellular carcinoma cell lines, E-cadherin, epithelial-mesenchymal transition

on cell proliferation and differentiation by promoting these potentials of undifferentiated cells (8). UPF1 also promotes the decay of mRNAs encoding several other proteins that oppose the proliferative and undifferentiated cell state (9). Our previous study reported that UPF1 was downregulated and its gene was commonly mutated in pancreatic adenocarcinoma (ASC), and to the best of our knowledge, it was the first to report UPF1 gene mutations in nonsense-mediated mRNA decay in ASC (10). However, a limited number of studies have reported the mechanism by which the expression levels of UPF1 affect HCC.

In the present study, it was demonstrated that UPF1 was significantly downregulated in HCC cell lines compared with in normal hepatocytes. Based on this finding, the expression levels of 19 key proteins associated with numerous signaling pathways were investigated in the presence of UPF1 overexpression (OE). In addition, the effects of UPF1 expression on the EMT process were analyzed by targeting the protein expression of E-cadherin, N-cadherin, Vimentin and Twist-related protein 1 (Twist). The findings of the present study may contribute to the development of HCC treatment in the future.

Materials and methods

Cell culture. The Huh-7 HCC cell line, HL-7702 normal hepatocytes and 293T normal cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in Minimum Essential medium with 10% fetal bovine serum (both Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C. HL-7702 normal hepatocytes and 293T normal cells were cryopreserved and thawed prior to analysis.

Polymerase chain reaction (PCR). DNA was extracted from 293T cells following transfection with the UPF1 lentiviral vector. The UPF1 gene was amplified using primers designed by Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, CA, USA) and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The primer sequences were as follows: UPF1 forward, 5'-AACACTATCAACCCG GGAGC-3' and reverse, 5'-GCTAGCTCATTTGTCGTC ATC-3'; GAPDH forward, 5'-TGACTTCAACAGCGACAC CCA-3' and reverse, 5'-CACCCTGTTGCTGTAGCCAAA-3'. The PCR reaction system contained 13.25 µl double-distilled water, 5 µl 10X PCR buffer (Mg²⁺ Plus), 2 µl deoxynucleotide triphosphates, 2 µl DNA template, 1 µl forward primer, 1 µl reverse primer and 0.25 µl rTaq DNA polymerase (all from Takara Bio, Inc., Otsu, Japan), in a total volume of 25 µl. The PCR thermocycling conditions were as follows: 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 1 min; and 72°C for 10 min. The PCR products were separated by 1.5% agarose gel electrophoresis. The positive control was GAPDH.

Reverse transcription-quantitative (RT-q)PCR. Total RNA was extracted from cells using SuperfecTRI containing TRIzol (Takara Bio, Inc., Otsu, Japan). cDNA was obtained following RT using the M-MLV kit (Promega Corporation), according to the manufacturer's protocols. qPCR was performed on

Step-one Plus system (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols of the SYBR-Green kit (Takara Biotechnology Co., Ltd., Dalian, China). The thermocycling conditions were as follows: Denaturation at 95°C for 10 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 20 sec. GAPDH was used as an internal control, and the 2^{-ΔΔC_q} method (11) was used to normalize expression levels against GAPDH. Each sample was analyzed in triplicate. The primers used in the present study were presented in Table I.

Lentiviral vector construction and transduction. UPF1 (GenBank: NM_002911; <https://www.ncbi.nlm.nih.gov/genbank/>) was cloned into GV358 vector (Ubi-MCS-3FLAG-SV40-EGFP-IRES-puromycin; Shanghai Genechem Co., Ltd., Shanghai, China) at restriction site AgeI. A total of 4x10⁵ 293T cells were transfected with 1 µg plasmid using the FuGene HD transfection reagent (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocols. Nontransduced cells (CON) and negative control cells transfected with the empty vector (NC) were used as the controls. Green fluorescent protein was used as a reporter, to measure transfection efficiency by confocal microscopy (magnification, x100). Four randomly chosen microscopic fields were analyzed. The lentiviral supernatant was harvested 48-72 h after transfection and used to infect Huh-7 cells at a viral titer of 2x10⁸ TU/ml. The expression levels of UPF1 were confirmed by RT-qPCR and western blotting (described below).

Western blotting. Protein was extracted from cells using radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology, Haimen, China) and protein concentration was determined by bicinchoninic acid assay. Cellular proteins (20 µg) from each sample were electrophoresed by SDS-PAGE (5% stacking and 10% separating gels). Then, the proteins were transferred onto polyvinylidene fluoride membranes (Merck KGaA, Darmstadt, Germany) and blocked with 5% skim milk in Tris-buffered saline and Tween-20 for 1 h at room temperature. The membranes were incubated with primary antibodies overnight at 4°C. Subsequently, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1.5 h at room temperature. All antibodies used in the present study were listed in Table II. Protein bands were visualized using Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific, Inc.). The positive control was the standard protein SURVIVIN-3FLAG-GFP (Shanghai Genechem Co., Ltd.).

Protein antibody array assay. The PathScan® Cancer Phenotype Antibody Array kit (Cell Signaling Technology, Inc., Danvers, MA, USA) was utilized to investigate alterations in the expression of key proteins associated with various signaling pathways in the presence of UPF1 overexpression. A total of 2x10⁵ cells were lysed using cell lysis buffer (cat. no. 7018; Cell Signaling Technology, Inc.) on ice, and then incubated with a slide for 2 h at room temperature or 4°C overnight. Following washing, the slide was incubated sequentially with detection antibody cocktail, horseradish peroxidase-linked streptavidin, and LumiGLO® and peroxide

Table I. Primer sequences employed in the present study.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')	Length (bp)
UPF1	CCTTGTGAGGGCAAAATGCAA	TGAAGCCGAGGAGGAAGACGT	113
GV358-UPF1	GAGGATCCCCGGGTACCGGTCGCCACCATGA GCGTGGAGGCGTACGGGGCCAGCTCGCAGA CTCTC	TCCTTGTAGTCCATACCATACTG GGACAGCCCCGTCACCCCGCC	3398
GAPDH	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA	121

UPF1, Up-frameshift 1.

Table II. Antibodies employed in the present study.

Antibody (cat. no.)	Dilution	Host species	Supplier
UPF1 (ab109363)	1:10,000-50,000	Rabbit	Abcam (Cambridge, UK)
P-UPF1 (07-1016)	1: 500	Rabbit	Merck KGaA
GAPDH (sc-32233)	1:2,000	Mouse	Santa Cruz Biotechnology, Inc. (Dallas, TX, USA)
FLAG (F1804)	1:3,000	Mouse	Sigma-Aldrich; Merck KGaA
E-cadherin (14472)	1:1,000	Mouse	Cell Signaling Technology, Inc.
N-cadherin (13116)	1:1,000	Rabbit	Cell Signaling Technology, Inc.
Vimentin (5741)	1:1,000	Rabbit	Cell Signaling Technology, Inc.
Twist (ab50581)	0.5-1.0 μ g/ml	Rabbit	Abcam
Goat anti-mouse IgG-HRP (sc-2005)	1:5,000	Goat	Santa Cruz Biotechnology, Inc.
Goat anti-rabbit IgG-HRP (sc-2004)	1:5,000	Goat	Santa Cruz Biotechnology, Inc.

HRP, horseradish peroxidase; IgG, immunoglobulin G; P-UPF1, phosphorylated Up-frameshift 1; Twist, Twist-related protein 1.

reagents. Images were obtained using a chemiluminescence scanner (ChemiScope 5300; Clinx Science Instruments Co., Ltd., Shanghai, China).

Statistical analysis. Data are presented as the mean \pm standard deviation from at least three independent experiments. A Student's t-test, χ^2 test or Fisher's exact test were used for the comparisons between groups using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

UPF1 is significantly downregulated in an HCC cell line. The expression levels of UPF1 were determined within HCC (Huh-7) and normal hepatocyte (HL-7702) cell lines. A significant downregulation of UPF1 in the HCC cell line was observed compared with in HL-7702 cells, as presented in Fig. 1. The expression levels of UPF1 in the HCC cell line was $\sim 60\%$ lower than that of normal hepatocytes ($P < 0.01$).

Successful transduction of the lentiviral vector constructs and UPF1 overexpression. Following transduction, the packaging cell line, 293T, exhibited notable green fluorescence (Fig. 2A). This indicated that the lentivirus was successfully transduced and that the UPF1 target gene was overexpressed. Expression

of the target sequence following total DNA extraction and subsequent PCR was observed. Furthermore, a band near 125 kDa was detected on the gel, whose location is consistent with the target protein observed via western blotting (Fig. 2B). The positive control (PC) was encoded by a scrambled sequence, independent of the UPF1 sequence.

UPF1 OE exhibits no effect on the levels of protein phosphorylation. Numerous studies have demonstrated that the phosphorylation of key proteins is involved in tumorigenesis (12,13). To investigate the association between UPF1 OE and the extent of phosphorylation, the levels of phosphorylated UPF1 protein (P-UPF1) following lentiviral transduction of Huh-7 HCC and 293T normal cells were determined. Prior to transduction, no target band was observed for P-UPF1 for Huh-7 cells; however, a weak target band was observed under the UPF1 OE condition in Huh-7 cells (Fig. 3A). Following over-exposure several nonspecific bands were observed; therefore, the P-UPF1 band in Huh-7 cells may also be considered nonspecific (Fig. 3A). To investigate the effects of lentiviral transduction on the HCC cell line, another set of experiments were conducted to transduce the HCC cell line for UPF1 OE. As presented in Fig. 3B, UPF1 and P-UPF1 were detected in Huh-7 cell. Compared with in the CON and NC cell groups, UPF1 was notably upregulated in the OE group. However, the multiple bands observed for P-UPF1 may be nonspecific.

UPF1 is involved in the regulation of several key proteins of numerous signaling pathways. The PathScan Cancer Phenotype Antibody Array kit was utilized to investigate the effects of UPF1 on the expression of numerous signaling pathways in Huh-7 HCC cells. All analyzed genes were listed in Table III; the results of chemiluminescent detection and gray-scale analysis were presented in Fig. 4A. Compared with in the NC groups, the expression levels of the majority of proteins were upregulated when UPF1 was overexpressed. The protein expression levels of cluster of differentiation (CD)31 (platelet endothelial cell adhesion molecule-1, PECAM-1), Vimentin, CD44, proliferating cell nuclear antigen (PCNA), Ki-67, N-Cadherin, retinoblastoma (Rb), Survivin, P53 and Met were significantly upregulated in their respective signal pathways (Fig. 4B; $P < 0.05$). These findings suggested that the expression levels of UPF1 exerted significant effects on numerous signaling pathways.

UPF1 serves an important role in EMT process. EMT is an important biological process for the development and progression of malignant tumors, in which epithelial cells are transformed to obtain malignant migration and invasive abilities (14,15). In the present study, 4 proteins (E-cadherin, N-cadherin, Vimentin and Twist) were selected as targets to investigate whether the expression levels of UPF1 can affect the EMT process in Huh-7 HCC cells. Western blotting revealed that compared with in the CON and NC groups, E-cadherin and N-cadherin were downregulated when UPF1 was overexpressed; however, that of Vimentin and Twist were notably upregulated (Fig. 5).

Overexpression of UPF1 affects the expression of antioncogene P53. P53 gene is a tumor suppressor gene (16,17). In the present study, the expression levels of P53 were investigated in the presence of UPF1 OE in Huh-7 HCC cell (Fig. 6). The results revealed that the expression of P53 was notably unaffected in the OE experimental group when UPF1 was overexpressed.

Discussion

HCC is the third leading cause of cancer-associated mortality worldwide (18). Several studies have demonstrated that tumorigenesis is a process associated with numerous factors (19-21). In the present study, the association between UPF1 expression and the EMT process underlying the tumorigenesis of HCC was investigated.

The expression levels of UPF1 in two cell lines were determined in the present study. RT-qPCR revealed that the expression levels of UPF1 in HCC cell lines were lower than that in normal hepatocytes, which was consistent with a recent report (22). This finding suggested a potentially important role of UPF1 in tumorigenesis. In addition, the phosphorylation posttranslational modification level of UPF1 was also evaluated in the presence and absence of UPF1 OE. Interestingly, no notable difference was observed in the OE group when UPF1 was overexpressed. Studies have reported that phosphorylation is one of the main factors associated with tumorigenesis (23-25); the present study reported notable association between the phosphorylation and overexpression of UPF1 in HCC.

To further understand the effects of UPF1 expression on the EMT process, a protein assay was conducted to determine

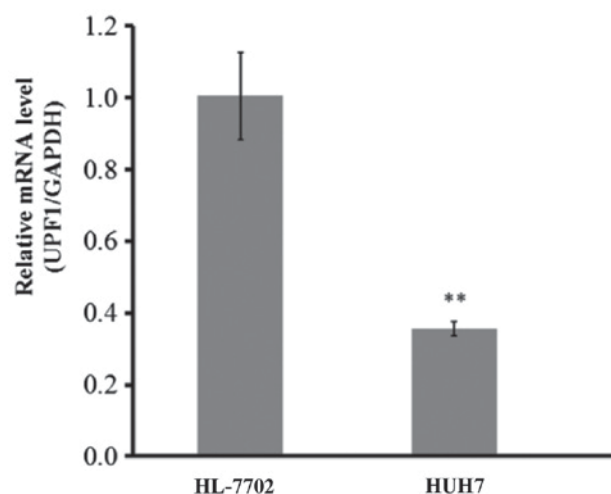


Figure 1. Downregulation of UPF1 expression levels in Huh-7 hepatocellular carcinoma cells and HL-7702 normal hepatocyte cells. ** $P < 0.01$ vs. HL-7702 cells. UPF1, Up-frameshift 1.

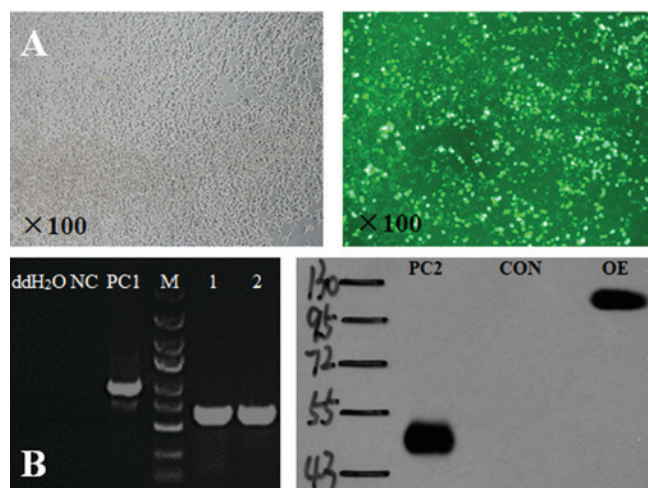


Figure 2. UPF1 gene lentiviral vector transduction and expression. (A) Analysis via confocal microscopy following transduction of 293T cell for UPF1 OE. (Left) bright field and (right) fluorescent field. Magnification, $\times 100$. (B) Gel electrophoresis (left) and western blotting (right) results for UPF1 expression. Lanes 1 and 2 are PCR products from 293T cells transfected with the UPF1 lentiviral vector. CON, nontransduced cells; NC, negative control; OE, overexpression; PC1, positive control 1 (GAPDH); PC2, positive control 2 (standard protein SURVIVIN-3FLAG-GFP); UPF1, Up-frameshift 1.

whether UPF1 OE affected the expression of key proteins of several signaling pathways. The majority of the proteins analyzed in the present study were upregulated in the presence of UPF1 OE. Among these proteins, CD31/PECAM-1, Vimentin, CD44, PCNA, Ki-67, N-cadherin, Survivin, p53, Met and Rb exhibited a significant association with UPF1 OE. Additionally, the expression levels of E-cadherin, of N-Cadherin and Vimentin were upregulated, which has been associated with the EMT process. Based on these findings, the present study investigated how UPF1 affects the EMT process. As aforementioned, the process of EMT serves an important role in embryonic development, chronic inflammation, tissue reconstruction, cancer metastasis and variety of fibrotic diseases, and possesses unique characteristics of gene regulation, such as that for E-cadherin downregulation (26,27). Western blotting

Table III. Protein array analysis results.

Target	Site	Modification	Average Gray Value		Standard deviation		P-value	Upregulated/ downregulated (%)
			NC	OE	NC	OE		
Positive control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CD31 (PECAM-1)	N/A	N/A	8.00	11.18	2.27	1.57	0.0181	39.79
EpCAM	N/A	N/A	33.52	37.78	9.04	3.17	0.3159	12.73
Vimentin	N/A	N/A	14.38	19.92	3.21	3.37	0.0155	38.47
CD44	N/A	N/A	8.05	10.70	1.99	1.44	0.0247	32.92
CD45	N/A	N/A	9.80	11.08	2.23	0.84	0.2169	13.10
PCNA	N/A	N/A	26.75	37.73	8.83	7.33	0.0410	41.06
Ki-67	N/A	N/A	41.90	55.62	5.60	6.16	0.0024	32.74
p27 Kip1	N/A	N/A	23.88	24.57	3.47	2.51	0.7043	2.86
E-Cadherin	N/A	N/A	47.90	56.78	8.21	6.69	0.0670	18.55
N-Cadherin	N/A	N/A	46.78	63.45	6.81	2.41	0.0012	35.63
VE-Cadherin	N/A	N/A	12.58	14.13	2.62	2.55	0.3235	12.32
MUC1	N/A	N/A	13.28	17.88	3.54	3.79	0.0550	34.63
Rb	Ser807/811	Phosphorylation	69.27	76.88	4.64	1.27	0.0089	11.00
HIF-1α	Total	N/A	13.18	16.32	2.93	2.83	0.0890	23.77
Survivin	Total	N/A	17.25	24.10	4.65	3.49	0.0163	39.71
p53	Total	N/A	27.05	33.82	3.48	3.86	0.0097	25.02
HER2/ErbB2	Total	N/A	6.50	6.75	1.73	1.69	0.8051	3.85
Met	Total	N/A	15.07	19.08	3.52	2.55	0.0473	26.66
EGF Receptor	Total	N/A	18.18	23.02	5.32	2.48	0.0716	26.58

Values in bold indicate proteins that were significantly different in OE vs. NC ($P < 0.05$). CD, cluster of differentiation; EGF, epidermal growth factor; EpCAM, epithelial cell adhesion molecule; HER2/ErbB2, human epidermal growth factor receptor 2; HIF-1 α , hypoxia inducible factor-1 α ; PECAM-1, platelet endothelial cell adhesion molecule-1; PCNA, proliferating cell nuclear antigen; MUC1, mucin 1; N/A, not applicable; NC, negative control; OE, overexpression; Rb, retinoblastoma protein; VE-cadherin, vascular endothelial cadherin.

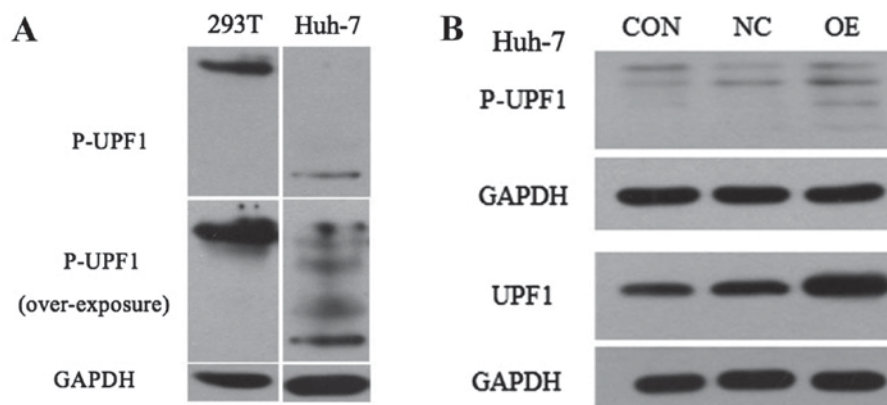


Figure 3. UPF1 OE exhibits no notable effects on the phosphorylation of UPF1. (A) Phosphorylation analysis of cells prior to lentiviral transduction. (B) Detection of UPF1 expression and phosphorylation following lentiviral transduction of the UPF1 gene. NC, negative control; CON, untransduced cells; OE, overexpression; P-UPF1, phosphorylated-Up-frameshift 1.

demonstrated the expression levels of N-cadherin, Vimentin and Twist were upregulated under the UPF1 OE condition, which was consistent with the findings of PathScan analysis of Huh-7 HCC cells. In addition, when UPF1 was overexpressed, the expression of E-cadherin was downregulated in the present study, which

was contradictory to the results of PathScan analysis. These observations suggested that UPF1 did not serve its role to suppress the expression of N-cadherin, Vimentin and Twist when overexpressed; the expression of E-cadherin may be regulated by the manifold. In a variety of human tumors, the role of E-cadherin

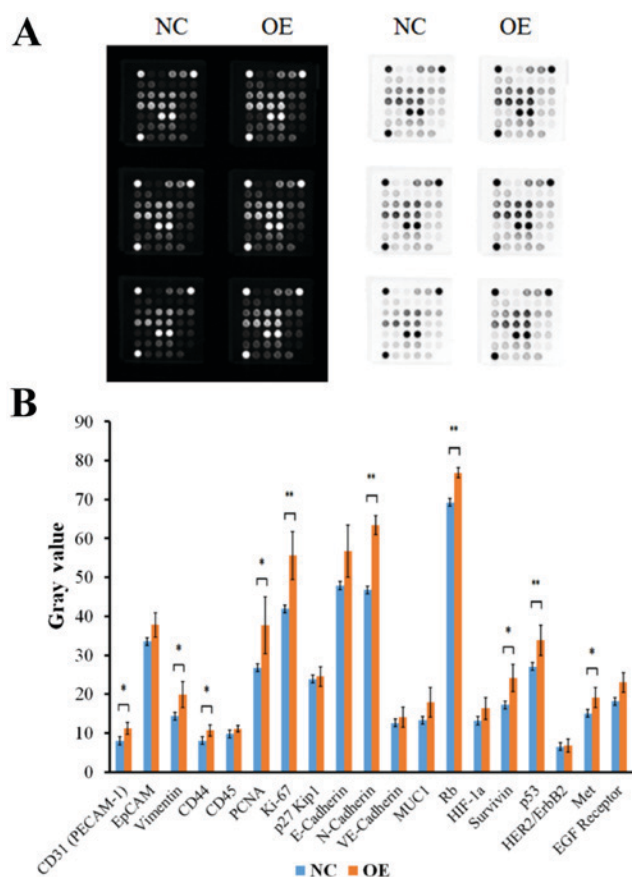


Figure 4. UPF1 OE upregulates the expression of numerous key proteins. (A) Results of chemiluminescent detection and gray analysis. (B) Compared with in the NC group the expression levels of the majority of proteins were upregulated when UPF1 was overexpressed. * $P < 0.05$, ** $P < 0.01$ vs. the NC group. CD, cluster of differentiation; EGF, epidermal growth factor; EpCAM, epithelial cell adhesion molecule; HER2/ErbB2, human epidermal growth factor receptor 2; HIF-1 α , hypoxia inducible factor-1 α ; PECAM-1, platelet endothelial cell adhesion molecule-1; PCNA, proliferating cell nuclear antigen; MUC1, mucin 1; NC, negative control; OE, overexpression; Rb, retinoblastoma protein; UPF1, Up-frameshift 1; VE-cadherin, vascular endothelial cadherin.

may be affected by gene mutations, resulting in mutant proteins, abnormal post-translational modification (phosphorylation and glycosylation) and increased protein hydrolysis (28). Conversely, the results of the present study indicated that higher expression levels of Twist associated with the overexpression of UPF1 may lead to the downregulation of E-cadherin and upregulation of N-cadherin, which is consistent with recent findings (29,30). At present, the molecular mechanism of UPF1 underlying the EMT process is notably complex and is not fully understood; however, the results of the present study suggested the possibility that the expression levels of UPF1 affect the EMT process by targeting E-cadherin, N-cadherin, Vimentin and Twist proteins.

Furthermore, the expression levels of P53 were determined when UPF1 was overexpressed in the present study. P53 has been reported as a tumor suppressor gene, and its inactivation may promote tumor formation (31). The results of the present study demonstrated that UPF1 OE did not affect the expression levels of P53 in Huh-7 HCC cell.

Several studies have demonstrated low expression levels of UPF1 in HCC cells and UPF1-suppressed tumorigenesis (32). Conversely, the results of RT-qPCR and western blotting in the

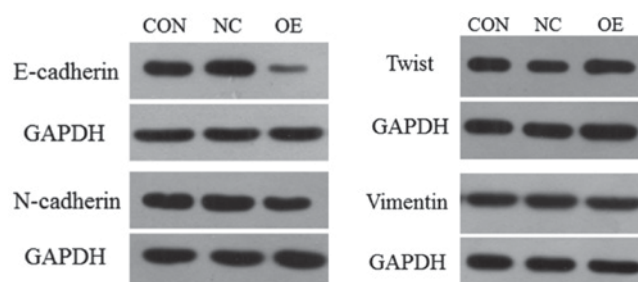


Figure 5. UPF1 expression levels affect the epithelial-mesenchymal transition process. Western blotting demonstrated that the expression levels of N-cadherin, Vimentin and Twist were upregulated under the UPF1 OE condition, but E-cadherin was downregulated. CON, untransduced cells; Twist, Twist-related protein 1; NC, negative control; OE, overexpression; UPF1, Up-frameshift 1.

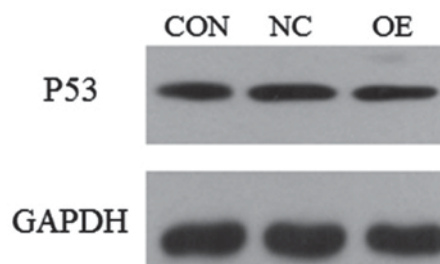


Figure 6. UPF1 does not affect the expression levels of P53. Western blotting demonstrated that the expression levels of P53 remained the same following UPF1 OE in Huh-7 cells. CON, untransduced cells; NC, negative control; OE, overexpression; UPF1, Up-frameshift 1.

present study revealed that UPF1 OE promoted the expression of numerous key proteins in several signaling pathways, including N-cadherin, Vimentin and Twist, contrary to the characteristics of UPF1; the expression of E-cadherin may be regulated by the manifold. The results of the present study indicated that UPF1 may be a potential target for regulating the EMT process (32).

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 81702450), the Natural Science Research Major Project of Education Office of Anhui Province (grant no. KJ2016SD40) and Support Program for Outstanding Young Talents in Colleges and Universities of Anhui Province (grant no. gxyq2018038).

Availability of data and materials

The datasets used or analyzed during the present study are available from the corresponding authors on reasonable request.

Authors' contributions

YL, QW and FS designed the study and drafted the manuscript. YL, TZ, YC and ZZ performed the experiments. SQ,

RW and YL analyzed and interpreted the data. All authors 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

- Klingenberg M, Matsuda A, Diederichs S and Patel T: Non-coding RNA in hepatocellular carcinoma: Mechanisms, biomarkers and therapeutic targets. *J Hepatol* 67: 603-618, 2017.
- Gong C, Fang J, Li G, Liu HH and Liu ZS: Effects of microRNA-126 on cell proliferation, apoptosis and tumor angiogenesis via the down-regulating ERK signaling pathway by targeting EGFL7 in hepatocellular carcinoma. *Oncotarget* 8: 52527-52542, 2017.
- Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer Statistics in China, 2015. *CA Cancer J Clin* 66: 115-132, 2016.
- Kuo TC, Chen CK, Hua KT, Yu P, Lee WJ, Chen MW, Jeng YM, Chien MH, Kuo KT, Hsiao M and Kuo ML: Glutaminase 2 stabilizes Dicer to repress Snail and metastasis in hepatocellular carcinoma cells. *Cancer Lett* 383: 282-294, 2016.
- Luo W, Zhu X, Liu W, Ren Y, Bei C, Qin L, Miao X, Tang F, Tang G and Tan S: MYC associated zinc finger protein promotes the invasion and metastasis of hepatocellular carcinoma by inducing epithelial mesenchymal transition. *Oncotarget* 7: 86420-86432, 2016.
- Yoshinaga T, Uwabe K, Naito S, Higashino K, Nakano T, Numata Y and Kihara A: AM251 suppresses epithelial-mesenchymal transition of renal tubular epithelial cells. *PLoS One* 11: e0167848, 2016.
- Kim CW, Hwang KA and Choi KC: Anti-metastatic potential of resveratrol and its metabolites by the inhibition of epithelial-mesenchymal transition, migration, and invasion of malignant cancer cells. *Phytomedicine* 23: 1787-1796, 2016.
- Azzalin CM and Lingner J: The human RNA surveillance factor UPF1 is required for S phase progression and genome stability. *Curr Biol* 16: 433-439, 2006.
- Lou CH, Shao A, Shum EY, Espinoza JL, Huang L, Karam R and Wilkinson MF: Posttranscriptional control of the stem cell and neurogenic programs by the nonsense-mediated RNA decay pathway. *Cell Rep* 6: 748-764, 2014.
- Liu C, Karam R, Zhou Y, Su F, Ji Y, Li G, Xu G, Lu L, Wang C, Song M, *et al*: The UPF1 RNA surveillance gene is commonly mutated in pancreatic adenocarcinoma. *Nat Med* 20: 596-598, 2014.
- 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.
- Carr MI, Roderick JE, Gannon HS, Kelliher MA and Jones SN: Mdm2 phosphorylation regulates its stability and has contrasting effects on oncogene and radiation-induced tumorigenesis. *Cell Rep* 16: 2618-2629, 2016.
- Takada M, Zhang W, Suzuki A, Kuroda TS, Yu Z, Inuzuka H, Gao D, Wan L, Zhuang M, Hu L, *et al*: FBW7 loss promotes chromosomal instability and tumorigenesis via cyclin E1/CDK2-mediated phosphorylation of CENP-A. *Cancer Res* 77: 4881-4893, 2017.
- Ma F, Li W, Liu C, Li W, Yu H, Lei B, Ren Y, Li Z, Pang D and Qian C: MiR-23a promotes TGF-β1-induced EMT and tumor metastasis in breast cancer cells by directly targeting CDH1 and activating Wnt/β-catenin signaling. *Oncotarget* 8: 69538-69550, 2017.
- Natsuizaka M, Whelan KA, Kagawa S, Tanaka K, Giroux V, Chandramouleeswaran PM, Long A, Sahu V, Darling DS, Que J, *et al*: Interplay between Notch1 and Notch3 promotes EMT and tumor initiation in squamous cell carcinoma. *Nat Commun* 8: 1758, 2017.
- Solomon H, Brosh R, Buginim Y and Rotter V: Inactivation of the p53 tumor suppressor gene and activation of the Ras oncogene: Cooperative events in tumorigenesis. *Discov Med* 9: 448-454, 2010.
- Rivlin N, Brosh R, Oren M and Rotter V: Mutations in the p53 tumor suppressor gene: Important milestones at the various steps of tumorigenesis. *Gene Cancer* 2: 466-474, 2011.
- Venook AP, Papandreou C, Furuse J and de Guevara LL: The incidence and epidemiology of hepatocellular carcinoma: A global and regional perspective. *Oncologist* 15 (Suppl 4): 5-13, 2010.
- Jin H, Li Q, Cao F, Wang SN, Wang RT, Wang Y, Tan QY, Li CR, Zou H, Wang D and Xu CX: miR-124 inhibits lung tumorigenesis induced by K-ras mutation and NNK. *Mol Ther Nucleic Acids* 9: 145-154, 2017.
- Furic L, Rong L, Larsson O, Koumakpayi IH, Yoshida K, Brueschke A, Petroulakis E, Robichaud N, Pollak M, Gaboury LA, *et al*: eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. *Proc Natl Acad Sci USA* 107: 14134-14139, 2010.
- Zhang X, Qiao Y, Wu Q, Chen Y, Zou S, Liu X, Zhu G, Zhao Y, Chen Y, Yu Y, *et al*: The essential role of YAP O-GlcNAcylation in high-glucose-stimulated liver tumorigenesis. *Nat Commun* 8: 15280, 2017.
- Chang L, Li C, Guo T, Wang H, Ma W, Yuan Y, Liu Q, Ye Q and Liu Z: The human RNA surveillance factor UPF1 regulates tumorigenesis by targeting Smad7 in hepatocellular carcinoma. *J Exp Clin Cancer Res* 35: 8, 2016.
- Darini C, Wang S, Krishnamoorthy J and Koromilas A: Translation initiation factor eIF2 serine 51 phosphorylation suppresses HER2-mediated tumorigenesis and increases sensitivity of HER2⁺ breast cancer to trastuzumab therapy. *Mol Cancer Res* 16: 82-83, 2018.
- Zhang Y, Yu G, Chu H, Wang X, Xiong L, Cai G, Liu R, Gao H, Tao B, Li W, *et al*: Macrophage-associated PGK1 phosphorylation promotes aerobic glycolysis and tumorigenesis. *Mol Cell* 71: 201-215.e7, 2018.
- Han T, Zhan W, Gan M, Liu F, Yu B, Chin YE and Wang JB: Phosphorylation of glutaminase by PKCε is essential for its enzymatic activity and critically contributes to tumorigenesis. *Cell Res* 28: 655-669, 2018.
- Dai X, Ahn KS, Wang LZ, Kim C, Deivasigamni A, Arfuso F, Um JY, Kumar AP, Chang YC, Kumar D, *et al*: Ascochlorin enhances the sensitivity of doxorubicin leading to the reversal of epithelial-to-mesenchymal transition in hepatocellular carcinoma. *Mol Cancer Ther* 15: 2966-2976, 2016.
- Zhang WN, Li W, Wang XL, Hu Z, Zhu D, Ding WC, Liu D, Li KZ, Ma D and Wang H: CLDN1 expression in cervical cancer cells is related to tumor invasion and metastasis. *Oncotarget* 7: 87449-87461, 2016.
- Ross JS, Wang K, Sheehan CE, Boguniewicz AB, Otto G, Downing SR, Sun J, He J, Curran JA, Ali S, *et al*: Relapsed classic E-cadherin (CDH1)-mutated invasive lobular breast cancer shows a high frequency of HER2 (ERBB2) gene mutations. *Clin Cancer Res* 19: 2668-2676, 2013.
- Balakrishnan S, Bhat FA, Raja Singh P, Mukherjee S, Elumalai P, Das S, Patra CR and Arunakaran J: Gold nanoparticle-conjugated quercetin inhibits epithelial-mesenchymal transition, angiogenesis and invasiveness via EGFR/VEGFR-2-mediated pathway in breast cancer. *Cell Prolif* 49: 678-697, 2016.
- Bokhari AA, Baker TM, Dorjbal B, Waheed S, Zahn CM, Hamilton CA, Maxwell GL and Syed V: Nestin suppression attenuates invasive potential of endometrial cancer cells by downregulating TGF-β signaling pathway. *Oncotarget* 7: 69733-69748, 2016.
- Du J, Wang Y, Chen D, Ji G, Ma Q, Liao S, Zheng Y, Zhang J and Hou Y: BAY61-3606 potentiates the anti-tumor effects of TRAIL against colon cancer through up-regulating DR4 and down-regulating NF-κB. *Cancer Lett* 383: 145-153, 2016.
- Zhang H, You Y and Zhu Z: The human RNA surveillance factor Up-frameshift 1 inhibits hepatic cancer progression by targeting MRP2/ABCC2. *Biomed Pharmacother* 92: 365-372, 2017.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.