Original Article
Aberrant USP11 expression regulates NF90 to promote proliferation and metastasis in hepatocellular carcinoma

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Abstract: Growing evidence indicates that deubiquitinase ubiquitin-specific protease 11 (USP11) plays an important role in cellular function by regulating the stability of its substrates. USP11 is dysregulated in many types of cancer and involved in tumor development and progression. We previously showed that USP11 was upregulated in hepatocellular carcinoma (HCC) and promoted HCC cell invasion and metastasis potency. However, the mechanism underlying the role of USP11 in HCC cell metastasis and its function in cell proliferation remain unknown. Here, CCK-8, soft agar assays and nude mouse models showed that USP11 was essential for HCC cells survival and proliferation in vitro and in vivo. Results from mass spectrometry, co-immunoprecipitation, and ubiquitination assays demonstrated that USP11 interacted with nuclear factor 90 (NF90) and promoted its deubiquitination, thereby stabilizing it in HCC cells. Moreover, the effect of USP11 on promoting HCC cells proliferation and metastasis was dependent on NF90, and USP11 expression was positively correlated with NF90 expression in human HCC tissues, as demonstrated via immunohistochemistry. Collectively, the present findings indicated that USP11 binded to and deubiquitinated NF90, thereby stabilizing the protein expression level and promoting HCC cell proliferation and metastasis. NF90 was identified as an important downstream target of USP11. Dysregulated signaling of this novel USP11/NF90 axis might promote HCC proliferation and metastasis, and the axis could be a potential therapeutic target in HCC.

Keywords: Hepatocellular carcinoma, ubiquitin-specific protease 11, nuclear factor 90, proliferation, metastasis

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide with a highly aggressive behavior [1]. Approximately 500-1000 new cases are diagnosed each year, of which >50% occur in China because of the prevalence of hepatitis B virus infection [2]. Despite advances in the early diagnosis of HCC and the availability of several treatment modalities, such as surgery, trans-arterial chemical embolization, liver radio frequency ablation, and liver transplantation, the prognosis for patients with HCC is still poor. This poor prognosis is attribute to the high rates of malignancy, invasiveness, and metastasis, the rapid progression, and the high postoperative recurrence rate of HCC [3, 4]. Therefore, elucidation of the mechanisms underlying HCC initiation, progression, and metastasis is important to prolong the survival of patients with HCC.

Ubiquitin-specific protease 11 (USP11), a member of the family of deubiquitinating enzymes, is primarily localized in the cell nucleus. USP11 plays an important role in signal transduction, apoptosis, DNA repair, and viral replication by regulating the stability of its substrates such as p53, PML, IκBα, and cIAP2 [5-8]. Consistent with its important regulatory role, USP11 is dysregulated in many types of cancer, including glioma [6], breast cancer [9, 10], and ovarian cancer [11]. USP11 regulates glioma pathogenesis by stabilizing PML, which is achieved via
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Antagonization of RNF4-facilitated PML ubiquitination [6]. USP11 can also promote epithelial mesenchymal plasticity and metastasis of breast cancer by deubiquitinating TGFBR2 [9]. In addition, upregulation of USP11 promotes the epithelial to mesenchymal transition by deubiquitinating Snail in ovarian cancer [11]. Our previous work showed that USP11 was upregulated in HCC and promoted HCC cell invasion and metastasis [12]. However, the mechanism underlying the role of USP11 in HCC cell metastasis and the biological function of USP11 in HCC cell proliferation remain to be determined.

Nuclear factor 90 (NF90), a double-stranded RNA binding protein, is a protein encoded by the interleukin enhancer-binding factor 3 gene. NF90 and its partner NF45 are involved in diverse cellular processes, including DNA-break repair [13], cell cycle regulation [14], and cell growth and proliferation [15]. NF90 also plays an important role in human cancer progression. NF90 binds to the 3' untranslated region of cyclin E1 mRNA, and depletion of NF90 delays cell-cycle progression, inhibits cell proliferation, reduces tumorigenic capacity, and sensitizes HCC cells to the cyclin-dependent kinase inhibitor roscovitine [14]. Moreover, the DPBP76/NF90 isoform facilitates vascular endothelial growth factor (VEGF) expression by stabilizing VEGF mRNA under hypoxia conditions, and promotes breast cancer angiogenesis and tumor progression [16]. In addition, suppression of NF90 in cervical cancer cell lines downregulates VEGF-A expression, thereby inhibiting angiogenesis and decreasing cellular tumorigenic capacity [17]. Although the biological function of NF90 has been extensively investigated, its role in HCC initiation, progression and its relationship with USP11 remain poorly understood.

Here, we showed for the first time that USP11, a novel NF90 interactor, directly promoted NF90 deubiquitination and stabilization. We also demonstrated that the USP11-NF90 axis was critical for regulating the proliferation and metastasis of HCC cells. Our findings revealed an important missing piece in the regulation of NF90 stability, and identified a previously unknown molecular function of USP11 in controlling HCC cell proliferation and metastasis. These results provided a novel mechanistic insight into the role of USP11 in HCC proliferation and metastasis, which could advance our knowledge of the molecular basis of HCC development, and indicated that the USP11-NF90 axis might be a new therapeutic target in HCC.

Materials and methods

Cell culture

The human HCC cell lines SMMC-7721, Huh7 and MHCC-97h were obtained from the cell bank of Shanghai Institute of Cell Biology, were cultured in DMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Millipore), and in a humidified incubator under 5% CO₂ at 37°C.

Plasmid construction and lentivirus preparation

For overexpression experiments, human complementary DNAs corresponding to full-length of USP11 and NF90 were obtained via reverse transcription PCR (RT-PCR) amplification. For USP11 knockdown, shRNA sequences were inserted into the pLV-shRNA-puro plasmid interference vector. The following target sequences for USP11 shRNAs were designed and chemically synthesized: shRNA-1: 5'-CCGGTGCCGTGACTACAACACTCTACTCGAGTAGGAGTTGTTGATGTCACGGTTTTTTG-3'; shRNA-2: 5'-CCGGTGCCGTGATGATATCTTCGTCTACTCGAGTAGACGAGATATCATCACGGTTTTTG-3'. The plasmids generated and the virus packaging plasmids were transfected into HEK-293T cells using TurboFect Transfection Reagent (Thermo, #R0531 and #R0532) according to the manufacturer's protocol. After 48 h, the viral supernatant was transfected into HEK-293T cells using TurboFect Transfection Reagent (Thermo, #R0531 and #R0532) according to the manufacturer's protocol. After 48 h, the viral supernatant was collected for infection of HCC cells, and the cells were selected with puromycin (1-2 mg/ml, Sigma) to construct stably infected cell lines.

RNA isolation and quantitative real-time PCR

Total RNA was extracted from the different HCC cell lines using TriPure Isolation Reagent (Roche) and cDNA was synthesized using a GoScript™ RT system kit (Promega, Madison, WI) according to the manufacturer’s instructions. After RT of total RNA, qPCR was performed to examine the expression levels of USP11 and NF90 using SYBR Green PCR Master mix (Takara Biotechnology, Ltd.) on a real-time PCR instrument (Bio-Rad, Carlsbad, CA, USA). GAPDH was used as an internal reference gene to normalize mRNA levels between...
different samples for exact comparison of transcription levels. The following primers sequences were used for quantitative real-time PCR: GAPDH, forward: 5'-CTTTGGTGATCGTGGAGGAAGTGT-3'; USP11, forward: 5'-CTTTCCCGGACCAGATACTACGTT-3'; reverse: 5'-CATCAGGGGAAGATTTGACGTT-3'; NF90, forward: 5'-AACCATGGAGGCTACATGAATAC-3'; reverse: 5'-CGCTCTAGGAAGACCAAAATC-3'. Datas were analyzed using the ΔΔCt method with GAPDH as the constitutive marker.

Western blotting

Proteins were extracted using RIPA lysis buffer (Beyotime) containing a phosphatase inhibitor (Roche) and a protease inhibitor (Sigma). Protein content was determined with the bicinchoninic acid kit (Thermo). Briefly, proteins were separated via 10% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore). The PVDF membranes were blocked with skim milk for at least 1 h at room temperature and then incubated with primary antibodies overnight at 4°C, followed by incubation with corresponding secondary antibody and visualization with ECL Western Blotting Substrate (Millipore). Western blot analysis was performed using the following antibodies: anti-β-actin (Cell Signaling Technology), anti-USP11, and anti-NF90 (Proteintech Group) antibody. The results were observed via chemiluminescence.

Cell viability analysis

To explore the effects of USP11 on cell proliferation, 2 × 10^3 cells were cultured in 96-well plates with 100 µl of DMEM using the cell counting Kit-8 (HY-K0301, Beijing) assay. Cells were then cultured for a further 1, 2, 3, or 4 days. Subsequently, all cells were incubated with 10% CCK-8 solution at 37°C for 4 h. The absorbance was measured at a 450 nm using a microplate spectrophotometer. All experiments were performed triplicately.

Plate clone formation assay

A total of 2 × 10^3 cells were cultured in 6-well plates and fixed with 4% paraformaldehyde for 30 min, and then stained with 1.0% crystal violet for 10 min until formed visible clones. The number of colonies was counted in 10 different fields.

Migration and invasion assays

8-µm pore size transwell plates (BD Biosciences) with or without Matrigel were placed into 24-well plates containing 500 µl of medium with 10% FBS. Cells were seeded at a density of 5 × 10^4 cells were seeded into the upper chamber with 200 µl of serum-free medium. After routine culture for 24-48 h, the matrix and cells in the upper chamber were removed with a cotton swab. Then the cells were fixed with 4% paraformaldehyde for 30 min, stained with crystal violet and finally counted under a light microscope.

Co-Immunoprecipitation (co-IP) assay

Protein was extracted using the method described above. Cell lysates, 50 µl of magnetic beads (Novex), and 1 µg of the indicated antibody were incubated overnight at 4°C. The mixture was then placed on a magnet and the solid material was removed from the supernatant and washed three times with a washing buffer. Loading buffer was added to the tube and heated for 10 min at 100°C. Then the immunoreactive complex was collected using a magnet and subjected to SDS-PAGE and immunoblotting analysis.

Protein half-life assays

Cells in the exponential growth period were treated with cycloheximide (10 µg/ml) for different times to block protein synthesis. The cells were harvested and subjected to immunoblot analysis using antibodies against NF90 and β-Actin to observe changes in protein levels.

Ubiquitination assay

For in vivo ubiquitination assays, cells were treated with 10 µM MG-132 for 12 h to block the proteolytic activity of the 26S proteasome complex. Then the cell lysate extracted from USP11 knockdown and over-expression HCC cells were co-immunoprecipitated with an anti-NF90 antibody, and the ubiquitination level of NF90 was tested using an anti-Ub antibody. For in vitro ubiquitination assays, Ub-HA conjugated NF90-Flag were purified from HEK-293T cells with protein G-agarose (Millipore, 16-266) incubated with anti-Flag antibody. The protein complexes containing GFP tagged USP11 were purified from HEK-293T cells with anti-GFP M2 affinity gel (Sigma) followed by eluted through 3
Flag peptides (Sigma). Then, Ub-HA-NF90 and the USP11 protein complexes were incubated for 1 h, at 37°C in the reaction buffer (50 mM Tris PH 7.5, 10 mM MgCl$_2$, 1 mM DTT, 100 mM NaCl, 1 mM ATP). After reaction, the NF90-Flag protein was purified and immunoblotted with antibodies against Ub.

Animal studies

In order to verify the in vitro experimental results, animal experiments were performed in which 6 $\times$ 10$^6$ cells were subcutaneously injected into nude mice at the animal experiment center of Xiamen University. The tumor volume was measured every 5 days and evaluated as follows: (length $\times$ width$^2$)/2 cm$^3$. After 25 days, the nude mice were sacrificed and tumor weights were measured for further analysis. Animal work was performed using an approved Institutional Animal Care and Use Protocol approved by Xiamen University.

Clinical sample collection

All clinical samples were obtained from the Chronic Liver Disease Biological Sample Bank, Department of Hepatobiliary Surgery, Zhongshan Hospital Xiamen University. Specimen collection was performed after obtaining informed consent from each patients, and the study was approved by the ethics committee of the hospital.

Immunohistochemistry (IHC)

Tissue was fixed with 10% formalin and embedded in paraffin and cut into 4-µm sections for immunohistochemical staining. The sections were stained with anti-USP11 (1:2000, Proteintech) and anti-NF90 (1:4000, Proteintech) antibodies at 4°C overnight, incubated with a secondary antibody for 30 min at room temperature, developed with diaminobenzidine, and stained with hematoxylin. The IHC reagents were purchased from Maixin Biotechnology (Fuzhou).

IHC evaluation

Two clinical pathologists blinded to the experimental data evaluated the IHC-stained sections. Any inconsistencies in immunostained section scores were re-evaluated by a third pathologist to obtain a final result. In brief, 100 cells were randomly counted in microscopic field at $\times$ 200 magnification and were classified into five groups according to the percentage of cells with positive nuclear staining as follows: 0 = negative; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; and 4 = $\geq76%$. Meanwhile, the nuclear staining intensity was categorized as follows: 0 = negative; 1 = pale yellow; 2 = medium yellow; and 3 = tawny. The proportion and intensity scores were then multiplied to obtain a total score for each sample.

Statistical analysis

Data were analyzed using SPSS 21.0 and GraphPad Prism 7 software packages. Results are expressed as the mean ± standard deviation. Statistical analysis was performed using the two-related samples Wilcoxon nonparametric test for comparing two different groups. Spearman rank correlation was used to examine possible correlations between USP11 and NF90 expression. P<0.05 was considered statistically significant.

Results

USP11 promotes proliferation of HCC cells in vitro and in vivo

USP11 acts as an oncogene in most malignant solid tumors, including HCC. We previously showed that USP11 served as a marker of poor prognosis and promoted metastasis in HCC [12]. To further elucidate the function of USP11 in HCC cells, SMMC-7721 and MHC-97h cell lines with USP11-knockdown were generated via lentivirus infection with two USP11 specific shRNAs; control cells were infected with lentivirus containing a scramble shRNA (Figure 1A). Then, the effects of USP11 on HCC cell survival and proliferation were examined using the CCK-8 assay and soft agar colony formation assay. The CCK-8 assay revealed that USP11 knockdown decreased cell viability (Figure 1B). Similar results were obtained in soft agar colony formation experiments, which showed that colony formation ability and number were decreased for USP11 knockdown cells (Figure 1C). Conversely, wild-type USP11 and USP11-Mut (Cys318 is replaced by Ala and without catalytic activity) were over-expressed in Huh-7 cells (Figure 1D). CCK-8 and colony formation assays demonstrated that elevated expression of wild-type USP11 led to a pro-proliferative effect in HCC cells, while the expression of
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Identification of NF90 as a novel downstream target regulated by USP11 in HCC cells

To investigate the mechanism underlying the USP11-mediated regulation of HCC cell proliferation and metastasis, downstream targets of USP11 were identified via mass spectrometry in SMMC-7721 and MHCC-97h HCC cell lines. Various proteins with differential expression were identified in USP11 knockdown cells compared with control cells. On the base of
that USP11 is a deubiquitinase, we focused on the downregulated proteins in USP11 knockdown cells. XIAP and p21, which are known as the target proteins of USP11 [18, 19], were among the proteins identified by mass spectrometry, further confirming the reliability of our experiments. Among all the validated USP11-regulated targets identified, NF90 was selected for further experiments on the basis of previous reports that NF90 regulates HCC cell cycle progression and growth via modulation of cyclin E1 mRNA stability [14]. Higuchi et al. showed that knockdown of NF90 or NF45 decreased HCC cell proliferation [20]. Therefore, we performed co-IP experiments to validate the mass spectrometry findings. The results showed that endogenous USP11 and NF90 combined with each other in HCC cells (Figure 2A). Next, a reciprocal co-IP assay was carried out in 293T cells to further define the interaction between exogenous USP11 with NF90 and revealed a similar result (Figure 2B). To further verify that USP11 regulated NF90, protein expression was examined in HCC cells transfected with USP11 shRNAs. The results showed that NF90 was downregulated in HCC cells transfected with shRNA against USP11 (Figure 2C). Meanwhile, to correlate the biological response with the mechanisms identified in the HCC cell system, NF90 protein levels in vivo were assessed via western blotting. As shown in Figure 2D, knockdown of USP11 significantly downregulated NF90 in transplanted tumor tissues. By contrast, elevated expression of wild-type USP11 led to significant up-regulation of NF90 protein levels in HCC cells. However, overexpression of USP11-Mut had no effect on NF90 expression levels (Figure 2E). Taken together, the results point to NF90 as a novel downstream target of USP11 in HCC.

**Ectopic expression of NF90 promotes HCC cell proliferation and migration in vitro**

On the basis of the findings that USP11 regulates NF90, we explored the functional role of NF90 in HCC cells. Hence, SMMC-7721 and MHC-97h cell lines overexpressing NF90 were generated using a stable NF90 overexpression plasmid (Figure 3A). Results for CCK-8 and soft agar colony formation assays (Figure 3B, 3C) showed that NF90 overexpression increased the cell proliferation rate and the number of clones. What’s more, the migration and invasion assays results suggested that NF90 positively regulated HCC cell metabolism (Figure 3D). Taken together, these results suggested that NF90 functions as an oncogenic molecule involved in HCC progression.

**The effect of USP11 in promoting HCC cell proliferation and metastasis depends on NF90**

To determine the biological significance of NF90 in USP11-mediated HCC cell proliferation and metastasis, we performed rescue experiments by reintroducing NF90 into HCC cells expressing USP11-shRNA. The efficacy of knockdown and reintroduction was validated via immunoblotting analysis (Figure 4A). In the functional experiments, cell counting and colony formation assays showed that NF90 significantly reversed the proliferation-promoting effects of USP11 (Figure 4B, 4C). Migration and invasion assays in HCC cells showed that overexpression of NF90 promoted cell migration in USP11 knockdown cells (Figure 4D). Taken together, these results demonstrated that the USP11-NF90 axis contributed to HCC cell proliferation and metastasis.

**USP11 inhibits the ubiquitination-mediated degradation of NF90 in HCC cells**

USP11 knockdown significantly downregulated NF90 as revealed by western blot analysis. In addition, treatment with the protein synthesis inhibitor CHX for different times downregulated NF90 expression in USP11 knockdown cells compared with control cells (Figure 5A), indicating that knockdown of USP11 decreased the half-life of NF90. Given that USP11 altered only protein and not mRNA levels of NF90 (Figure S1), we hypothesized that USP11 regulated NF90 protein stability, but not its synthesis or transcription. Previous studies demonstrated that ubiquitination-mediated proteasomal degradation is a major mechanism in protein degradation. Hence, SMMC-7721 and MHCC-97h cells were treated with the proteasome inhibitor MG132. Western blot analysis showed that MG132 rescued USP11-mediated NF90 downregulation (Figure 5B). Wide type USP11 led to an increase in NF90 protein levels, but USP11-Mut failed to stabilize NF90 in HCC cells. Hence, ubiquitination assays were performed to explore whether USP11 was involved in the regulation of NF90 ubiquitination. The results showed that levels of NF90 ubiquitination were higher in USP11 knock-
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Figure 2. NF90 is a novel downstream target regulated by USP11 in HCC cells. A. Co-IP experiments indicated that USP11 interacted with NF90 in SMMC-7721 and MHCC-97h cells. Endogenous USP11 protein was immunoprecipitated with an anti-USP11 antibody and then analyzed via immunoblotting (upper panel). Endogenous NF90 protein was immunoprecipitated with an anti-NF90 antibody and then analyzed via immunoblotting (lower panel). An IgG antibody was used as the control. B. A co-IP assay was performed in 293T cells to explore the interaction between exogenous USP11 and NF90. C. NF90 expression levels in control and USP11 knockdown HCC cells were determined via western blotting. D. Western blot analysis of the expression of USP11 and NF90 proteins in SMMC-7721-shUSP11 and SMMC-7721-shCtrl tumors. E. Expression of USP11 and NF90 proteins in Huh-7 cells were detected via western blotting.
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By contrast, overexpression of USP11 dramatically decreased the level of NF90 ubiquitination in Huh7 cells (Figure 5D). Moreover, in vitro ubiquitination assays also showed that USP11 could significantly decreased NF90 ubiquitination levels (Figure 5E). Collectively, these results demonstrated that NF90 is a novel downstream target of USP11 and was regulated via USP11 through the ubiquitin degradation pathway in HCC cells.

Relationship between USP11 and NF90 in HCC clinical samples

To determine the relationship between USP11 and NF90 expression levels in human clinical HCC samples, USP11 and NF90 expression patterns were examined in a HCC tissue array using IHC. As shown in Figure 6A, NF90 levels were frequently increased in HCC tissues with high USP11 expression levels. Statistical analysis indicated a positive correlation between USP11 and NF90 in the 84 HCC samples examined (Figure 6B). Taken together, these data confirmed USP11 and NF90 co-expression pattern in HCC tissues and further validated the existence of a USP11-NF90 regulatory axis in HCC.

Discussion

USP11, a member of the deubiquitinate family, is highly expressed in many tumors and plays important roles in disease development and malignant progression. Invasion and metastasis contribute to the poor outcomes for
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B

![Graph showing OD Values (96 hours) for SMMC-7721 and MHCC-97h.](image)

C

![Images of cell culture plates for SMMC-7721 and MHCC-97h with control, ShUSP11, Control+NF90, and ShUSP11+NF90 conditions.](image)

D

![Images of migration and invasion assays for SMMC-7721 and MHCC-97h with control, ShUSP11, Control+NF90, and ShUSP11+NF90 conditions.](image)
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Figure 4. USP11 promotes HCC cells proliferation and metastasis dependents on NF90. A. The efficacy of knockdown and reintroduction was validated via immunoblotting analysis. B. NF90 abolished the proliferation inhibition induced by USP11 knockdown in HCC cells as confirmed by CCK-8 assays. C. NF90 abolished the proliferation inhibition induced by USP11 knockdown in HCC cells as confirmed by a colony formation assay. D. NF90 impaired the migration and invasion inhibition induced by USP11 knockdown in HCC cells as confirmed by migration and invasion assays.
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Figure 5. USP11 inhibits the ubiquitination-mediated degradation of NF90 in HCC cells. A. Control and USP11 knockdown HCC cells were treated with CHX for time intervals indicated. The cell lysates were subjected to immunoblotting (left panel) and NF90 expression level was quantified (right panel). B. MG132 treatment prevented USP11 knockdown-mediated NF90 degradation. C. Control and USP11 knockdown HCC cells were treated with MG132 10 µM for 8 h. Cell lysates were then subjected to immunoprecipitation with an anti-NF90 antibody. The resulting immunocomplexes were immunoblotted with anti-NF90 and anti-ubiquitin antibodies. D. Overexpression of USP11-wt not the USP11-mut dramatically decreased the level of NF90 ubiquitination in Huh7 cells. E. In vitro ubiquitination assays also showed that USP11-wt could significantly decreased NF90 ubiquitination levels.

Figure 6. USP11 expression is positively correlated with NF90 in clinical HCC tissues. A. Representative images of IHC staining of USP11 in HCC tissues with different expression levels of NF90. B. Spearman’s correlation analysis showed that USP11 expression was positively correlated with NF90 ($r = 0.3416, P = 0.0015$).

patients with HCC. Our previous research reveals that USP11 is upregulated in HCC tissues and cell lines. Statistical analyses demonstrates that USP11 upregulation is correlated with vascular invasion, differentiation, tumor number, and recurrence, as well as poor prognosis in HCC patients. Moreover, USP11 depletion suppresses HCC cell invasion and metastasis in vivo and in vitro [12]. However, the functional role of USP11 in HCC cell proliferation and the mechanism underlying the role of USP11 in HCC cell metastasis remain to be determined. In the current study, we demonstrated for the first time that USP11 could promote HCC cell proliferation, which was consistent with previous studies in lung epithelial cells [21] and osteosarcoma cell lines [22].

Together with the results of previous study, our present findings indicated that USP11 promoted the malignant features of HCC cells. However, the underlying molecular mechanism remains to be fully established. Here, SMMC-7721 and MHC-97h HCC cell lines were analyzed using mass spectrometry to identify possible downstream targets regulated by USP11. Considering the role of USP11 as a deubiquitinating enzyme, we focused on the downregulated proteins in USP11 knockdown cells. Jiang et al. showed that depletion of NF90 delays cell-cycle progression, inhibits HCC cell proliferation, and reduces tumorigenic capacity [14]. Higuchi et al. reached a similar conclusion, showing that knockdown of NF90 decreases the HCC cells proliferation [20]. Moreover, knockdown of NF90 also reduce tumor growth and angiogenesis in a cervical cancer cell line and a mouse xenograft model [17]. Therefore, we focused on NF90 in the present study. Rescue experiments showed that USP11 promoted HCC cells proliferation and metastasis in a manner dependent on NF90.

Previous work by our group showed that USP11 is high expressed in HCC tissues and mainly located in the cell nucleus. Analysis of NF90 protein expression in clinical specimens from the Human Protein Atlas (www.proteinatlas.org) revealed that NF90 was also high expressed in HCC cell nuclear. Similarly, NF90 is also overexpressed in human cervical cancer specimens and located in the nucleus [17]. These findings led us to hypothesize that USP11 and NF90 interact with each other in the cell nucleus.
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Surprisingly, results of Co-IP experiments confirmed this hypothesis and further validated the mass spectrometry findings. Increasing evidence indicates that USP11 binds to several substrates, thereby mediating their stabilization and deubiquitination. We found that USP11 regulated NF90 protein expression at the post-transcriptional level, and USP11 increased NF90 protein stability. We therefore assumed that USP11 could increase the stability of NF90 by decreasing its ubiquitination levels. We performed ubiquitination assays and showed that USP11 not only combined directly with NF90, but also dramatically decreased its ubiquitination levels. This regulatory mechanism resulted in increased NF90 protein levels in HCC cells, which suggested that USP11 may be a deubiquitinase for NF90. Finally, we explored USP11 and NF90 expression patterns in clinical HCC samples via IHC. The results suggested that USP11 was positively correlated with NF90 in clinical tissues, which was consistent with the results obtained at the cellular level.

In summary, the present study is the first time to demonstrate that USP11 directly binds to NF90 and promotes its deubiquitination, which increases its stability and further promotes cell proliferation and metastasis in HCC. The present study highlights the essential role of NF90 in the USP11-driven progression of HCC. The identification of a novel USP11/NF90 axis suggests a potential therapeutic strategy for HCC patients in the future.

Acknowledgements

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Disclosure of conflict of interest

None.

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Figure S1. USP11 altered only protein and not the mRNA levels of NF90. NF90 mRNA expression levels in control and USP11 knockdown HCC cells were determined using real-time PCR. **P<0.01; ***P<0.001; NS: not significant.