Original Article

FAM83D promotes the proliferation and migration of hepatocellular carcinoma cells by inhibiting the *FBXW7/MCL1* pathway

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Background: Hepatocellular carcinoma (HCC) is one of the most malignant tumors and has a poor 5-year survival rate. Family with sequence similarity 83, member D (*FAM83D*) is characterized as an oncogenic gene related to cell proliferation in many tumors, but the role and underlying mechanism of *FAM83D* in the development of HCC are still unclear.

Methods: *FAM83D* expression profiles and clinicopathological data were obtained from The Cancer Genome Atlas-Liver Hepatocellular Carcinoma (TCGA-LIHC). Additionally, 2 data sets from the Gene Expression Omnibus (GEO) database were used to further validate the *FAM83D* profile in HCC. We then downregulated the expression of *FAM83D* in HCC cells transfected with *FAM83D* small-interfering ribonucleic acid (siRNA) and upregulating its expression by *FAM83D*-overexpression transfection for further *in vitro* studies.

Results: TCGA and the GEO databases showed that *FAM83D* was significantly more upregulated in tumor tissues than non-tumor tissues. The high expression of *FAM83D* in HCC is associated with poor prognostic clinical factors. The knockdown of *FAM83D* in SNU449 and HUH7 cells *in vitro* impaired cell proliferation and migration, and promoted apoptosis, while the overexpression of *FAM83D* in BEL7402 cells had the opposite effect. Further, combined transfection with *FBXW7* siRNA or *MCL1*-overexpression reversed the role of *FAM83D* knockdown on cell proliferation, migration, and apoptosis *in vitro*, while *FBXW7* expression was negatively correlated with both the *FAM83D* and *MCL1* levels in TCGA-LIHC patients.

Conclusions: *FAM83D* played a significant role in HCC progression by enhancing cell proliferation and migration and inhibiting apoptosis, which may have been caused by the inhibition of the *FBXW7/MCL1* signaling pathway. Thus, *FAM83D* may be a promising therapeutic target for HCC.

Keywords: *FAM83D*; hepatocellular carcinoma (HCC); *FBXW7/MCL1*

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Introduction

Hepatocellular carcinoma (HCC), which accounts for >90% of primary tumors of the liver, is a highly prevalent tumor worldwide with rapidly increasing prevalence and mortality rates (1,2). The major risk factors of liver cirrhosis, including long-term virus infection, autoimmune hepatitis, and chronic alcohol use, are the common reasons for the development and progression of HCC. The morbidity and mortality of HCC remain high despite numerous interventions to prevent virus infection, reduce alcohol intake, and the like.

At present, surgery and interventional surgery are the main methods for treating HCC (3). However, the incidence of postoperative recurrence and metastasis is still high, and the 5-year survival rate (~12.5%) for patients with advanced HCC remains bleak (2). Additionally, while many researchers have studied the molecular mechanisms of HCC progression, due to its complexity, its specific pathogenesis has not yet been fully elucidated. Thus, it is crucial to further investigate the pathogenesis involved in the development and progression of HCC and thus develop promising and effective therapeutic strategies.

This research examined family with sequence similarity 83, member D (*FAM83D*), which is located on chromosome 20 encoding mitotic spindle-associated proteins (4). In lung cancer, *FAM83D* overexpression is associated with tumor size, lymph node metastasis, and TNM staging (5). *FAM83D* is also highly expressed in several cancers, such as ovarian cancer and metastatic lung adenocarcinoma (6,7). High *FAM83D* levels are associated with a poor clinical prognosis in colorectal cancer (8). However, the role and mechanism of *FAM83D* in the development of HCC have not been closely studied.

F-box and WD repeat domain containing 7 (*FBXW7*) is a member of the F-box family of proteins, and it is considered to be a tumor suppressor (9,10). *FBXW7* targets various mammalian proteins, regulates cell proliferation, growth, and apoptosis, and promotes proteasome degradation. *FBXW7* expression has been reported to be downregulated in patients with HCC (11), but the mechanism by which this occurs has not yet been closely investigated. A study has confirmed that the overexpression of *FAM83D* determines the alternative mechanism of *FBXW7* inactivation in breast cancer (6). Similarly, *FAM83D* knockout can cause the degradation of neurogenic locus notch homolog protein 1 (Notch1) by causing the upregulation of *FBXW7* (12,13). *MCL1* is one of the *FBXW7* substrates. *FBXW7* interacts with *MCL-1* and degrades through phosphorylation. *FBXW7* can regulate the malignant potential of cholangiocarcinoma and cisplatin-induced apoptosis through *MCL1*. Thus, we hypothesized that *FAM83D* might affect the development and progression of HCC by downregulating the *FBXW7* level and its related pathway.

There are now rich public repositories of ribonucleic acid (RNA)-sequencing and microarray data sets, such as The Cancer Genome Atlas (TCGA) (14) and the Gene Expression Omnibus (GEO) (15) databases. The current study sought to explore the effect of *FAM83D* on HCC development and progression. We found that the expression of *FAM83D* was significantly increased in tumor tissues and was associated with poor clinical prognosis in the TCGA-Liver Hepatocellular Carcinoma (TCGA-LIHC) data set. Then, we downregulated *FAM83D* expression in SNU449 and HUH7 cells transfected with *FAM83D* small interfering RNA (siRNA) and upregulated *FAM83D* expression by FMA83D overexpression transfection in BEL7402 cells. The ability of cell proliferation, migration, and apoptosis was evaluated. Additionally, *FAM83D* knockdown combined with *FBXW7* siRNA or *MCL1* overexpression transfection was performed to investigate the correlation between *FAM83D* and the *FBXW7*/*MCL1* pathway. *FBXW7* expression was found to be negatively correlated with both the *FAM83D* and *MCL1* levels in TCGA-LIHC data set. Our study revealed a new molecular pathogenesis of HCC. Our findings highlight a novel mechanism for *FAM83D* promotes HCC cell migration and invasion through *FBXW7*/*MCL1* signaling pathway. Therefore, it may be a potential new therapeutic target for HCC. We present the following article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2069/rc).

Methods

Bioinformatics analysis

TCGA

TCGA (http://cancergenome.nih.gov/) database was used to obtain the *FAM83D* expression profile and clinicopathological characteristics of TCGA-LIHC patients. A Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html) was conducted to analyze and visualize the data from the tumor and non-tumor samples from TCGA database and the Genotype-Tissue Expression (GTEx) project. Pathological

staging and related prognostic analyses were performed to examine differences in the transcription levels of *FAM83D* between tumor and non-tumor tissues. We used Kaplan-Meier curves for the prognostic analysis. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and the relevant policies in China.

GEO

Data sets with non-tumor tissues and HCC liver tumor tissues were obtained from the GEO database. The search keywords were "human", "liver", and "HCC". Ultimately, GSE25097 and GSE76427 from the GEO repository were included in the analysis. R software was used to compare the expression levels of *FAM83D* between tumor tissues and non-tumor tissues.

Cell culture and transfection

Human HCC cell lines (i.e., 7721, SNU449, BEL7402, Hep3b, and HUH7) were purchased from Nanjing Cobioer Biosciense Company. *FAM83D* siRNA (i.e., si*FAM83D*-1, si*FAM83D*-2, and si*FAM83D*-3), *FBXW7* siRNA (si*FBXW7*), siRNA negative control (siNC), *FAM83D* overexpression plasmid pcDNA-*FAM83D* (pcDNA-*FAM83D*), *MCL1* overexpression plasmid pcDNA-*MCL1* (pcDNA-*MCL1*), and pcDNA negative control (pcDNA-NC) were purchased from Genechem Company (Shanghai, China). Lentivirus particles were used to infect the SNU449, HUH7 and BEL7402 cells. After the cells were transfected for 24 h, the cells were collected for further experiments.

RNA extraction, semi-quantitative and real-time PCR

We used TRIZOL reagent (Invitrogen) to extract total RNA from cell culture samples, and reverse transcriptions were performed. The amplification was achieved by using SYBR green master mix through LightCycler 480. The following primers were used: *FAM83D*, forward: 5'-GGGAAGGTTCACGAAAAGT-3', reverse, 5'-GGCCAGACAGAATTACCAA-3'; β-actin: forward: 5'-CATGTACGTTGCTATCCAGGC-3', reverse, 5'-CTCCTTAATGTCACGCACGCT-3'.

Cell proliferation assays

We used 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays (Yuanye Company) to assess cell proliferation. We cultured the SNU499, HUH7, and BEL7402 cells in 96-well culture plates of Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal bovine serum. When the cells were 50% confluent, we transfected the cells with the siRNAs or pcDNA. After transfection, 20 μL of MTS solution was added to each well and incubated for 4 h. The optical density value was measured at 492 nm with a microplate reader. We used an EDU-based DNA cell proliferation kit (Beyotime Company) to detect cell proliferation and performed all the experiments according to the manufacturers' guidelines.

Wound healing assays

We cultured the HCC cells in 6-well plates. The cells were scratched with a 200-μL sterile pipette tip, washed 3 times with DPBS, and cultured for 24 h. Photographs were taken at 0 and 24 h after focusing on the same position.

Cell apoptosis

The transfected cells were stained with annexin V-PE and 7-AAD in accordance with the manufacturer's protocol, resuspended in phosphate buffered solution at room temperature for 30 min, and analyzed by flow cytometry (BD Company). The percentage of apoptosis in the cells was detected by flow cytometry. The apoptosis-related proteins were then examined by western bot.

Western blot

The total protein of the cells was dissolved in RIPA lysis buffer (Servicebio Company) containing a 10% protease inhibitor cocktail. We used the standard bicinchoninic acid method to detect the protein concentration of the lysate supernatant. Next, we performed a western blot analysis. Anti-human *FAM83D* (1:1,000), anti-Bcl-2, anti-Bax, anti-PARP, anti-CAS3, anti-E-CAD, anti-N-CAD, anti-VIM, anti-*FBXW7*, anti-*MCL1*, and β-actin (1:5,000) primary antibodies were used. Finally, we used Image J software for the relative protein analysis.

Statistical analysis

We used SPSS 20.0 statistical software for the statistical test. The data were presented as the mean ± standard deviation (SD). Statistical analyses between two groups were performed using the Student's *t*-test One-way analysis of

variance (ANOVA) was used to determine the significance of differences between groups. A P value <0.05 (2-tailed) was considered statistically significant.

Results

FAM83D was upregulated in HCC tumor tissues

FAM83D has been confirmed to be significantly upregulated in multiple human cancer types. To better explore the role of *FAM83D* in HCC, we analyzed TCGA database and found that *FAM83D* was significantly overexpressed in TCGA-LIHC tissues compared to adjacent non-tumor tissues (see *Figure 1A*). We then further explored the GEO database to verify the *FAM83D* expression profile in HCC. As expected, we found that *FAM83D* was significantly more upregulated in HCC tumor tissues than adjacent tissues (see *Figure 1B*).

FAM83D was correlated with poor clinical outcomes

To investigate the effect of *FAM83D* elevation on clinical outcomes, we also evaluated the association between the expression of *FAM83D* and the clinical characteristics of TCGA-LIHC patients. A high level of *FAM83D* was significantly associated with a higher clinical stage and tumor grade (see *Figure 1C*,*1D*). The higher expression TCGA-LIHC group of *FAM83D* was more likely to have a higher grade and stage.

We then examined the relationship between *FAM83D* and HCC survival using the cBioPortal for Cancer Genomics. The Kaplan-Meier estimator was used to analyze the prognosis of TCGA-LIHC patients based on *FAM83D* expression. In *Figure 1E*, the blue line represents LIHC with relatively low expression of *FAM83D*, and the red line represents LIHC with high expression of *FAM83D*. We found that TCGA-LIHC patients with higher levels of *FAM83D* had significantly lower overall survival (pHR =0.0042, log-rank P=0.0046).

In short, our findings provide further evidence that *FAM83D* overexpression can be used as a poor prognosis indicator in HCC. Next, we detected *FAM83D* expression in the following 5 HCC cell lines: 721, SNU449, BEL7402, Hep3b, and HUH7.

FAM83D promoted HCC cell proliferation

The Western blot analysis showed that *FAM83D* expression was high in the SNU449 and HUH7 cells, and lowest in BEL7402 cells (see *Figure 1F*). We then downregulated the expression of *FAM83D* in the SNU449 and HUH7 cells transfected with *FAM83D* siRNA and upregulating its expression in the BEL7402 cells transfected with *FMA83D* overexpression (see *Figure 1G-1I*). We used 2 *FAM83D* siRNA sequences with efficient transfection for the subsequent *in vitro* studies, and the *FAM83D* protein expression level was significantly reduced in the SNU449 and HUH7 cells transfected with si-*FAM83D*-1 and si-*FAM83D-2* (see *Figure 1G*,*1H*). Compared to the pcDNA-NC transfected cells, the *FAM83D* protein expression level was significantly increased after transfection with pcDNA-*FAM83D* in the BEL7402 cells (see *Figure 1I*).

To examine the role of *FAM83D* in the progression of HCC, we used MTT assays to evaluate the effects of *FAM83D* on the proliferation of HCC cell lines. Compared to the si-*FAM83D*-NC group, cell proliferation was significantly suppressed in the si-*FAM83D*-1 and si-*FAM83D*-2 groups. Conversely, cell proliferation was significantly enhanced following the overexpression of *FAM83D* (see *Figure 2A*).

The EDU proliferation assays also showed that *FAM83D* knockdown strongly reduced cell proliferation (see *Figure 2B*,*2C*). We then tested the effect of *FAM83D* overexpression in the BEL7402 cells, and found that cell proliferation was significantly enhanced following the overexpression of *FAM83D* (see *Figure 2D*). Further, we observed that the colony formation ability of the si-*FAM83D*-1 and si-*FAM83D*-2 groups was significantly decreased compared to the *FAM83D*-NC group (see *Figure 3A*). Compared to the pc-NC group, the number of colonies of the BEL7402 cells transfected with pcDNA-*FAM83D* increased significantly (see *Figure 3A*). Taken together, these results indicated that *FAM83D* played an important role in accelerating HCC cell proliferation.

FAM83D regulated cell migration

It is well known that migration is a sign of tumor metastasis. We performed an *in vitro* scratch-healing experiment to explore the effect of *FAM83D* on cell migration. Consistent with our expectations, *FAM83D* knockdown reduced wound closure and uncoated migration through the Boyden chamber. We then evaluated the effect of *FAM83D* overexpression on cell migration in the HCC cells. Similarly, we found that the overexpression of *FAM83D* accelerated wound closure and uncoated migration through

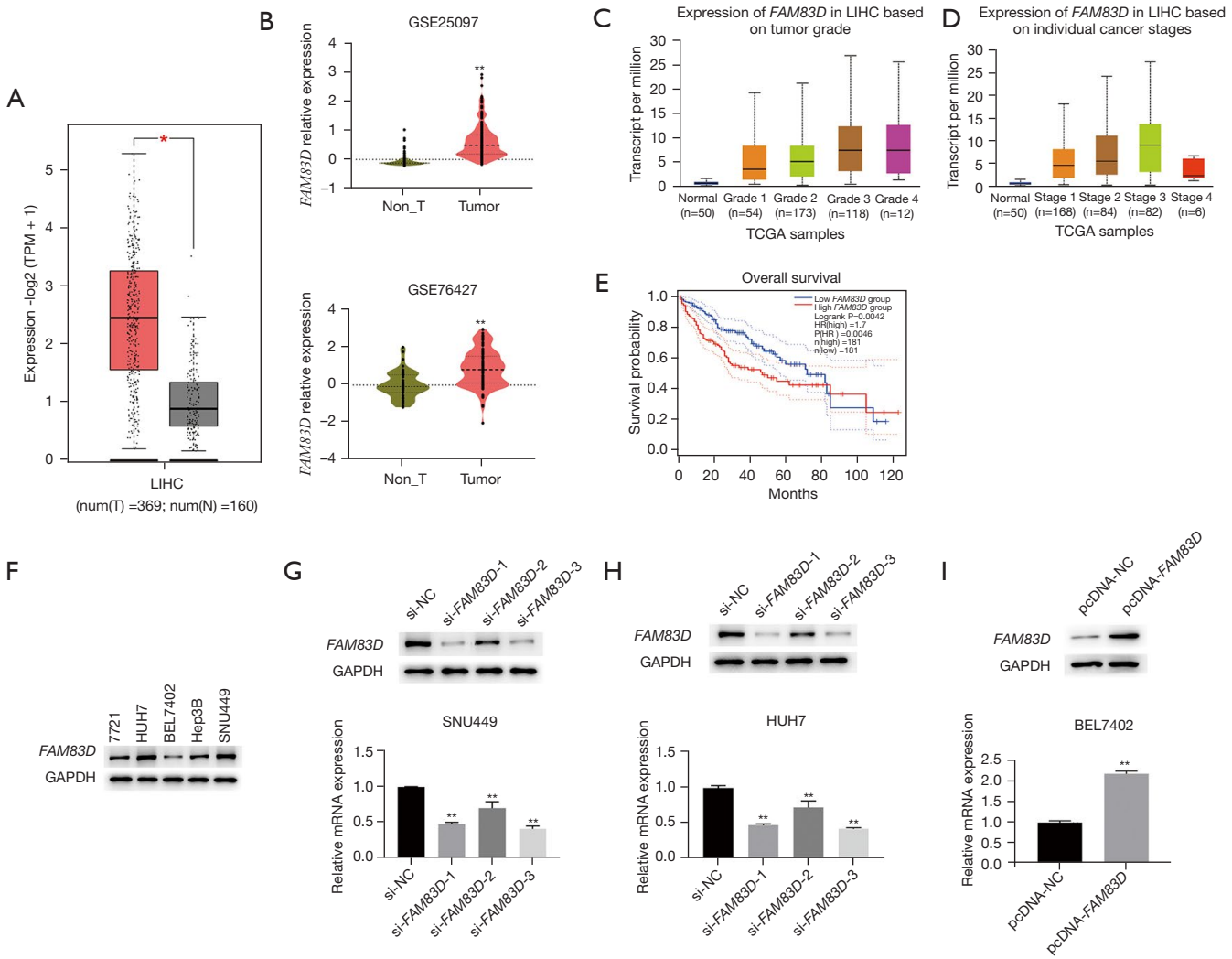

Figure 1 *FAM83D* is highly expressed in tumor tissues and is associated with patient prognosis. (A) The expression of *FAM83D* was higher in TCGA-LIHC tumor tissues than non-tumor tissues. (B) Data in GEO GSE25097 and GSE76427 show that the level of *FAM83D* is higher in HCC tissues than non-tumor tissues. (C) The expression of *FAM83D* in TCGA-LIHC tissues based on tumor grade. (D) The expression of *FAM83D* in TCGA-LIHC tissues based on individual cancer stages. (E) Patients with high levels of *FAM83D* had significantly lower overall survival than patients with low levels of *FAM83D*. (F) *FAM83D* protein expression in 5 HCC cell lines. (G) The effect of siRNA knockdown on *FAM83D* in SNU449 cells was verified by Western blotting. (H) The effect of siRNA knockdown on *FAM83D* in HUH7 cells was verified by Western blotting. (I) The effect of pcDNA overexpress on *FAM83D* in BEL7402 cells was verified by Western blotting. *P<0.05; **P<0.01. TPM, transcripts per million; TCGA-LIHC, The Cancer Genome Atlas-Liver Hepatocellular Carcinoma; GEO, Gene Expression Omnibus; HCC, hepatocellular carcinoma.

the Boyden chamber (see *Figure 3B*). Additionally, the western blotting analysis showed that the migration protein levels were significantly downregulated in the SNU449 and HUH7 cells following *FAM83D* knockdown. The migration protein levels were also significantly upregulated in the BEL7402 cells following *FAM83D* overexpression (see *Figure 3C*). These results indicated that *FAM83D* promoted cell migration in HCC.

FAM83D repressed apoptosis in HCC

We further explored the effect of *FAM83D* on the apoptosis

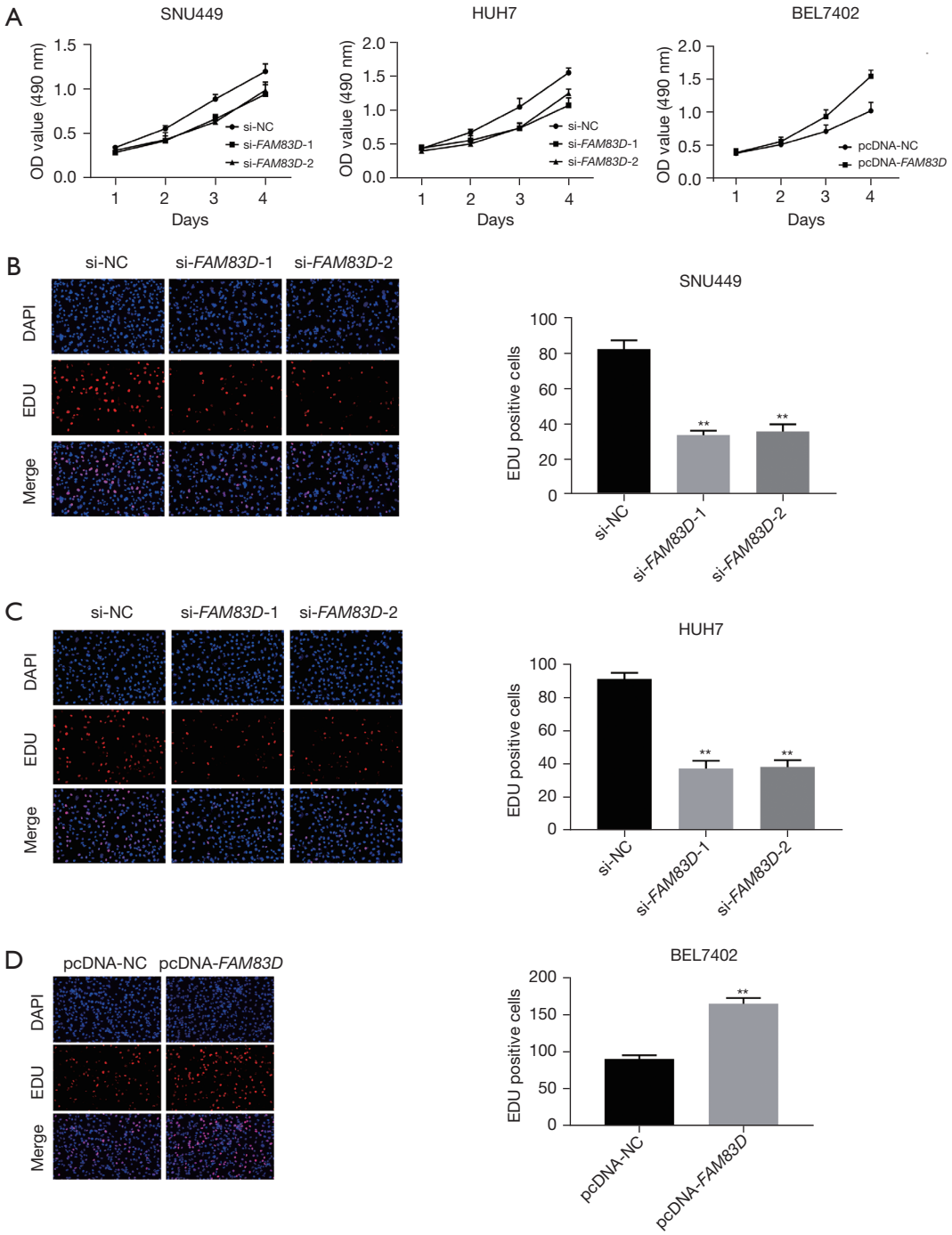

Figure 2 *FAM83D* promotes the proliferation of HCC cells *in vitro*. (A) MTT assays were used to assess the viability of *FAM83D* knockdown in HCC cells. (B,C) *FAM83D* knockdown suppressed the proliferation of SNU449 and HUH7 cells as measured by the EDU analysis (200×). (D) The overexpression of *FAM83D* promoted the proliferation of BEL7402 cells as measured by the EDU analysis (200×). **P<0.01. HCC, hepatocellular carcinoma; OD, optical density.

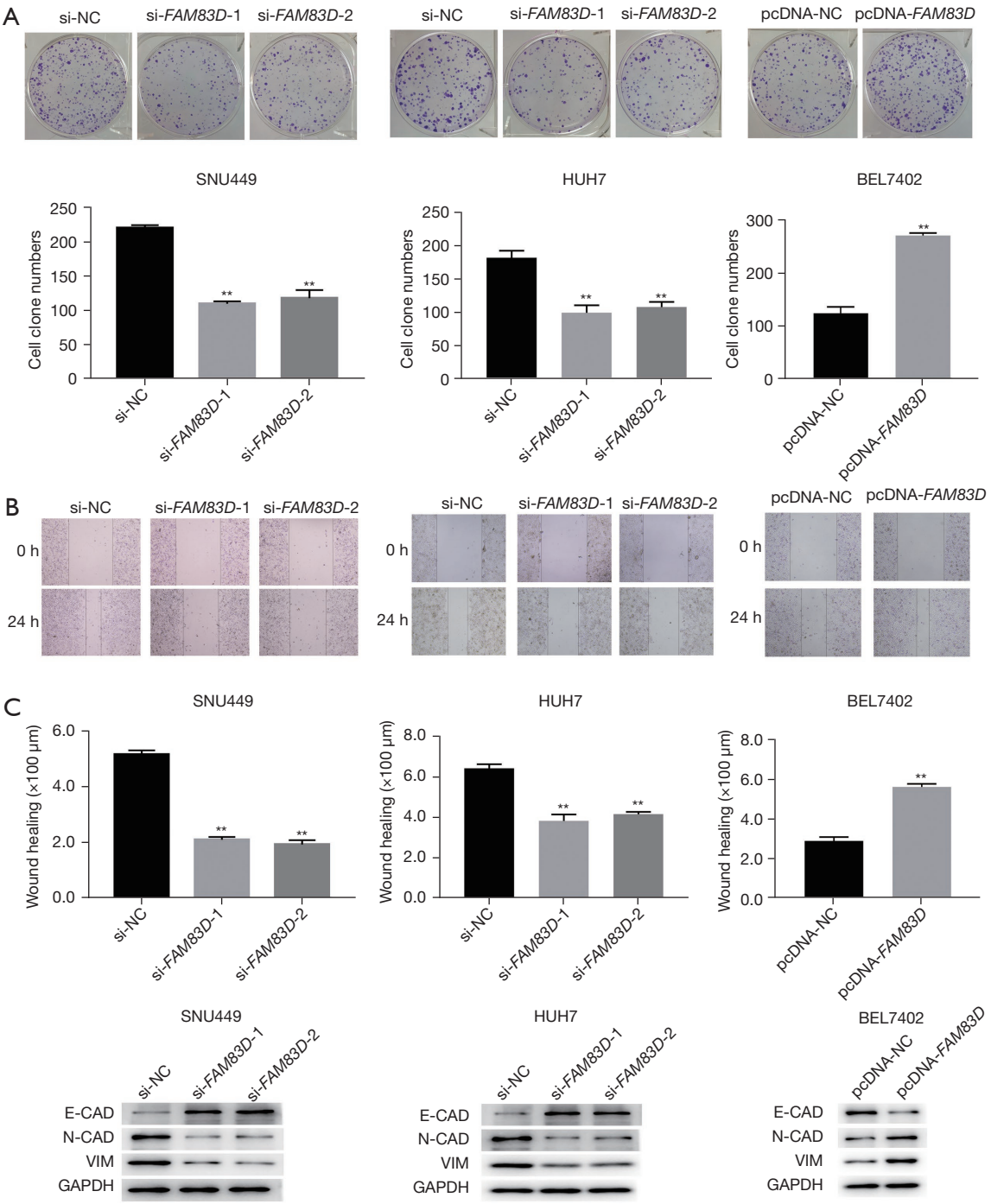

Figure 3 *FAM83D* promotes migration and invasion of HCC cells *in vitro*. (A) Colony formation experiments of control and *FAM83D* knockdown and overexpressed HCC cells. Top: representative images; bottom: quantification of colony numbers (crystal violet staining). (B) *FAM83D* knockdown suppressed SNU449 and HUH7 cell migration ability, and the overexpression of *FAM83D* promoted BEL7402 cell migration ability as measured by the scratch-healing experiment (40×). (C) Western blot analysis showed that the migration protein level of knocked down *FAM83D* cells was significantly downregulated, while the migration protein level of over expressed *FAM83D* cells was significantly upregulated. **P<0.01. HCC, hepatocellular carcinoma.

of HCC cells. The annexin V-PE flow cytometry results showed that *FAM83D* knockdown promoted early cell apoptosis in the SNU449 and HUH7 cells. Additionally, the western blot analysis indicated that *FAM83D* knockdown significantly reduced the protein expression level of Bcl-2 but increased the protein expression of Bax and PARP (see *Figure 4A*,*4B*). Similarly, the flow cytometry analysis showed that high levels of *FAM83D* expression inhibited cell apoptosis (see *Figure 4C*). We also confirmed that the overexpression of *FAM83D* downregulated the expression of apoptotic-related proteins (see *Figure 4C*). Taken together, these results revealed that *FAM83D* repressed apoptosis, which might lead to the progression of HCC. Next, we examined the mechanisms of the role of *FAM83D* in the progression of HCC.

FAM83D enhanced in-vitro cell proliferation, migration and suppressed apoptosis by inhibiting the FBXW7/MCL1 pathway

Recently, a study showed that the overexpression of *FAM83D* determined the alternative mechanism of *FBXW7* inactivation in breast cancer (6). Similarly, *FAM83D* knockout caused the degradation of Notch1 by causing the upregulation of *FBXW7* (12). We thus hypothesized that the malignant behavior of HCC cells caused by the overexpression of *FAM83D* might be related to the downregulation of *FBXW7*.

First, western blots were used to examine the expression levels of *FBXW7* and *MCL1* in HCC cells. The results showed that *FAM83D* knockdown significantly increased *FBXW7* expression but decreased the protein expression level of *MCL1* (see *Figure 5A*). Conversely, the overexpression of *FAM83D* in BEL7402 had the opposite effect to that described above (see *Figure 5A*). These results suggested that *FAM83D* might promote HCC progression by affecting the *FBXW7/MCL1* signaling pathway.

To further examine the effect of *FAM83D* on *FBXW7/MCL1* signal transduction in HCC cells, the HCC cells were co-transfected with si-*FAM83D* and si-*FBXW7* or with si-*FAM83D* and pc-*MCL1*. We found that either *FBXW7* knockdown or *MCL1* overexpression reduced the inhibitory effect of the downregulation of *FAM83D* on cell proliferation (see *Figure 5B*). Additionally, the scratch-healing experiment showed that the combination of *FBXW7* knockdown or *MCL1* overexpression reduced the inhibitory effect of *FAM83D* knockdown on cell migration (see *Figure 5C*). The Western blot analysis showed that both *FBXW7* knockdown and *MCL1* overexpression reversed the effect of reduced *FAM83D* expression on the migration protein level (see *Figure 6A*). Further, annexin V-PE flow cytometry and the western blot analysis showed that apoptosis was inhibited after the combined transfection with *FBXW7* siRNA or *MCL1* overexpression as compared to the only *FAM83D* siRNA group (see *Figure 6B*,*6C*). Thus, *FBXW7* knockdown or *MCL1* overexpression reduced the inhibitory effect of *FAM83D* knockdown on cell proliferation and migration, and the promotion of cell apoptosis. Our results indicated that *FAM83D* might regulate the proliferation and migration of HCC cells by inhibiting the *FBXW7/MCL1* signaling pathway.

Discussion

HCC is characterized by a high incidence, a high degree of malignancy, and an extremely low survival rate. Surgical techniques and radiotherapy and chemotherapy are common treatment options for cancer patients, and there has been some progress in the targeted therapy of HCC; however, HCC continues to have a high risk of recurrence and a poor prognosis. In recent years, people have become more and more interested in identifying new genes that play a key role in the initiation and progression of cancer. An analysis of TCGA database identified a significantly increased level of *FAM83D* in HCC tissues, and revealed that *FAM83D* was closely related to the clinical prognosis of TCGA-LIHC patients. The *FAM83D* expression profile was then verified in the GEO database. *In vitro* studies showed that *FAM83D* facilitated the cell proliferation and migration and inhibited the cell apoptosis of HCC cells. We also found that *FAM83D* promoted the progression of HCC by inhibiting the *FBXW7/MCL1* signaling pathway. These results suggested that *FAM83D* might be a promising target for HCC treatment.

FAM83D is located in the spindle and regulates the maintenance of the spindle, the process of mitosis, and cell division during mitosis (16,17). Studies have shown that the overexpression of *FAM83D* increases the migration and invasion of epithelial cells, which is related to a poor prognosis in breast cancer, lung cancer, and some other cancers (5,12). In our study, we demonstrated that *FAM83D* was an important molecule enhancing the proliferation and metastasis of HCC.

The analyses of TCGA and the GEO databases showed *FAM83D* was more upregulated in the HCC tumor tissues than the non-tumor tissues, and that a high level of

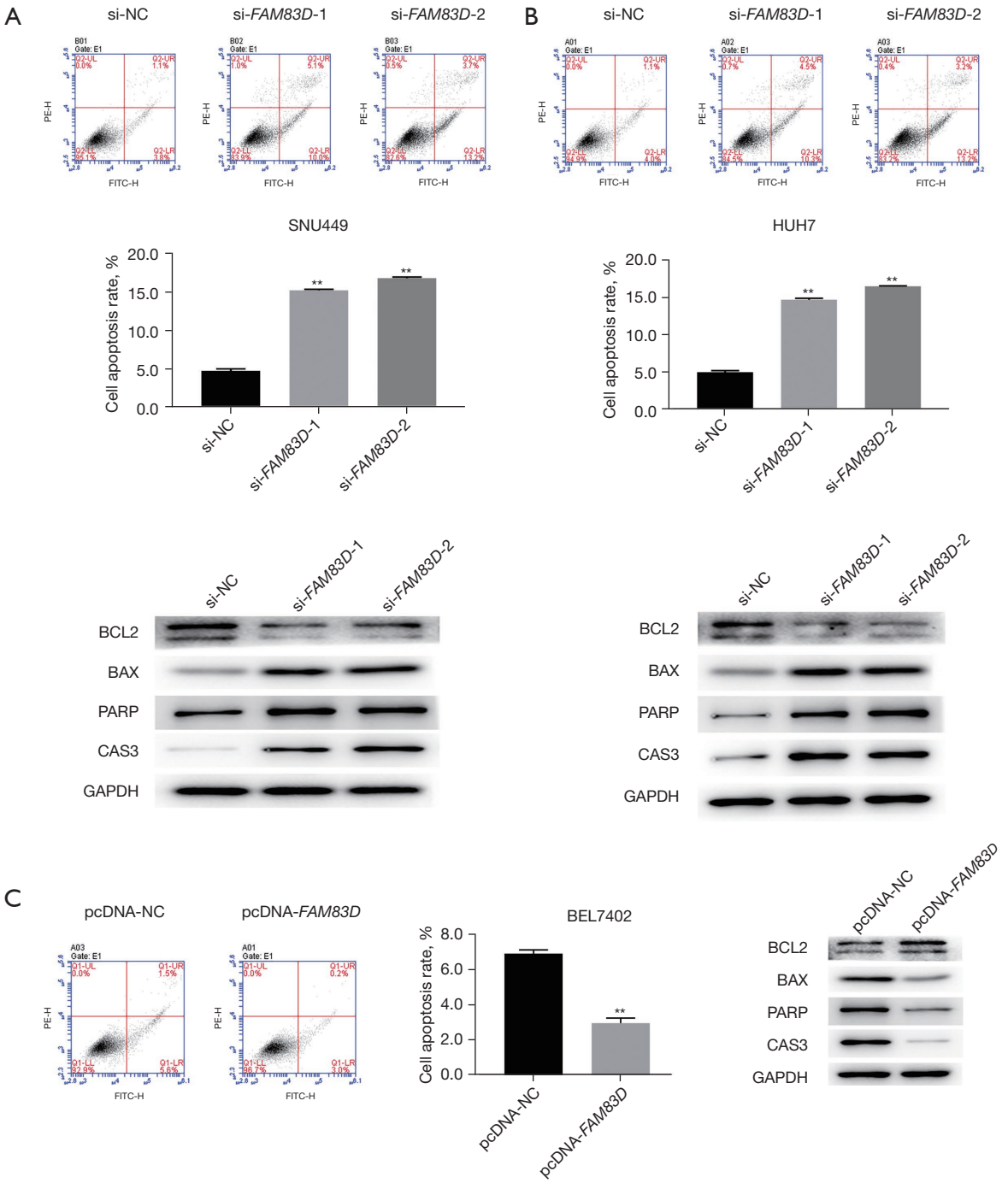

Figure 4 *FAM83D* attenuate the apoptosis of HCC cells *in vitro*. (A,B) Flow cytometry results showed that *FAM83D* knockdown promoted cell apoptosis and the apoptotic-related protein level as measured by western blot analysis. (C) Flow cytometry results showed that *FAM83D* overexpression inhibited cell apoptosis and the apoptotic-related protein level as measured by western blot analysis. **P<0.01. HCC, hepatocellular carcinoma.

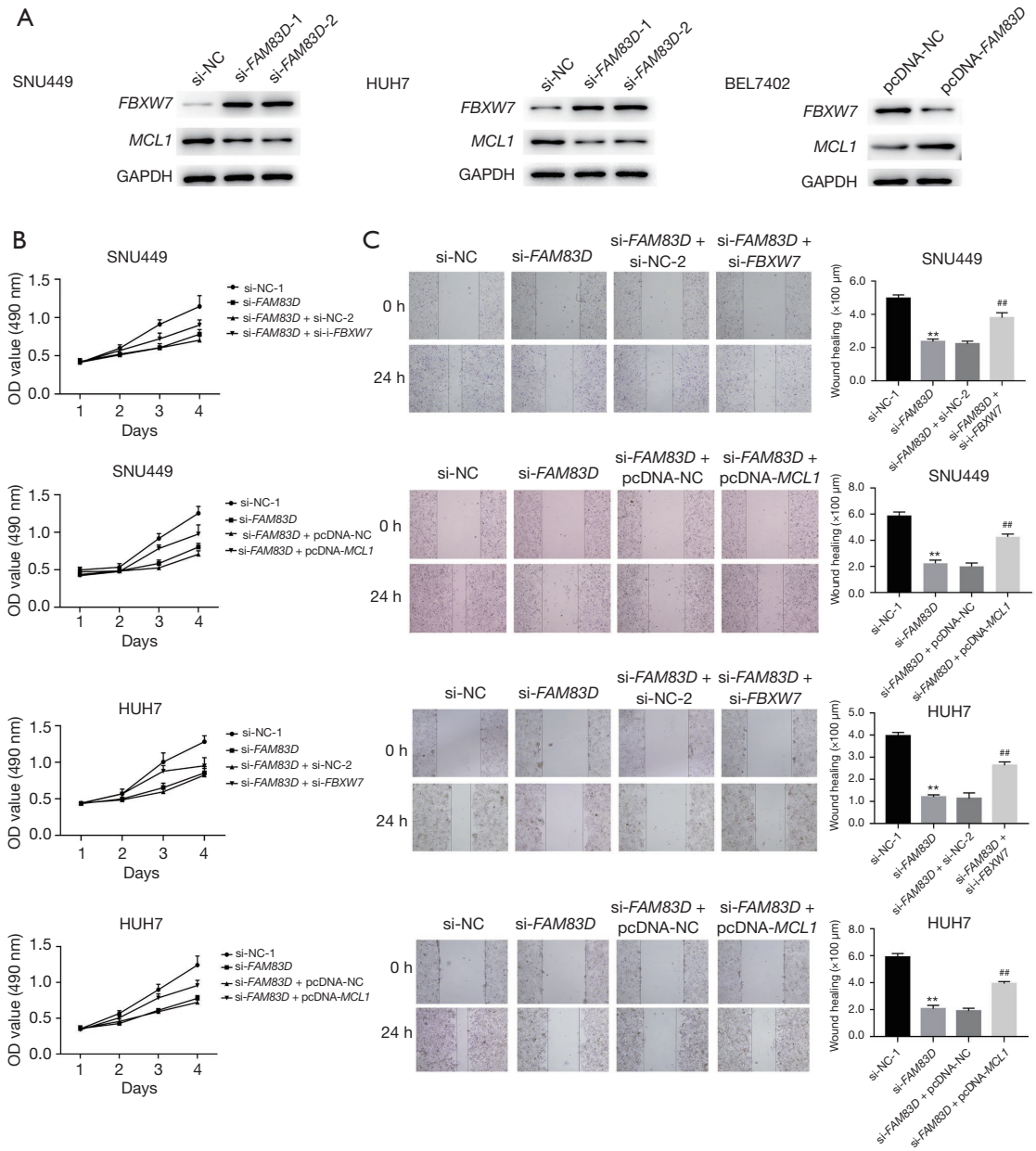

Figure 5 *FAM83D* activates *FBXW7*/*MCL1* pathway in HCC cells *in vitro* (HCC cells transfected with *FAM83D* siRNA; si*FAM83D* + si*FBXW7*; si*FAM83D* + pc*MCL1*). (A) The expression levels of *FBXW7* and *MCL1* proteins in HUH7 and SNU449 cells when *FAM83D* was knocked down as measured by a western blot analysis. (B) Cell proliferation was measured by MTT assays. (C) Cell migration ability was measured by scratch-healing experiments (40×). **P<0.01; ##P<0.01; HCC, hepatocellular carcinoma; OD, optical density.

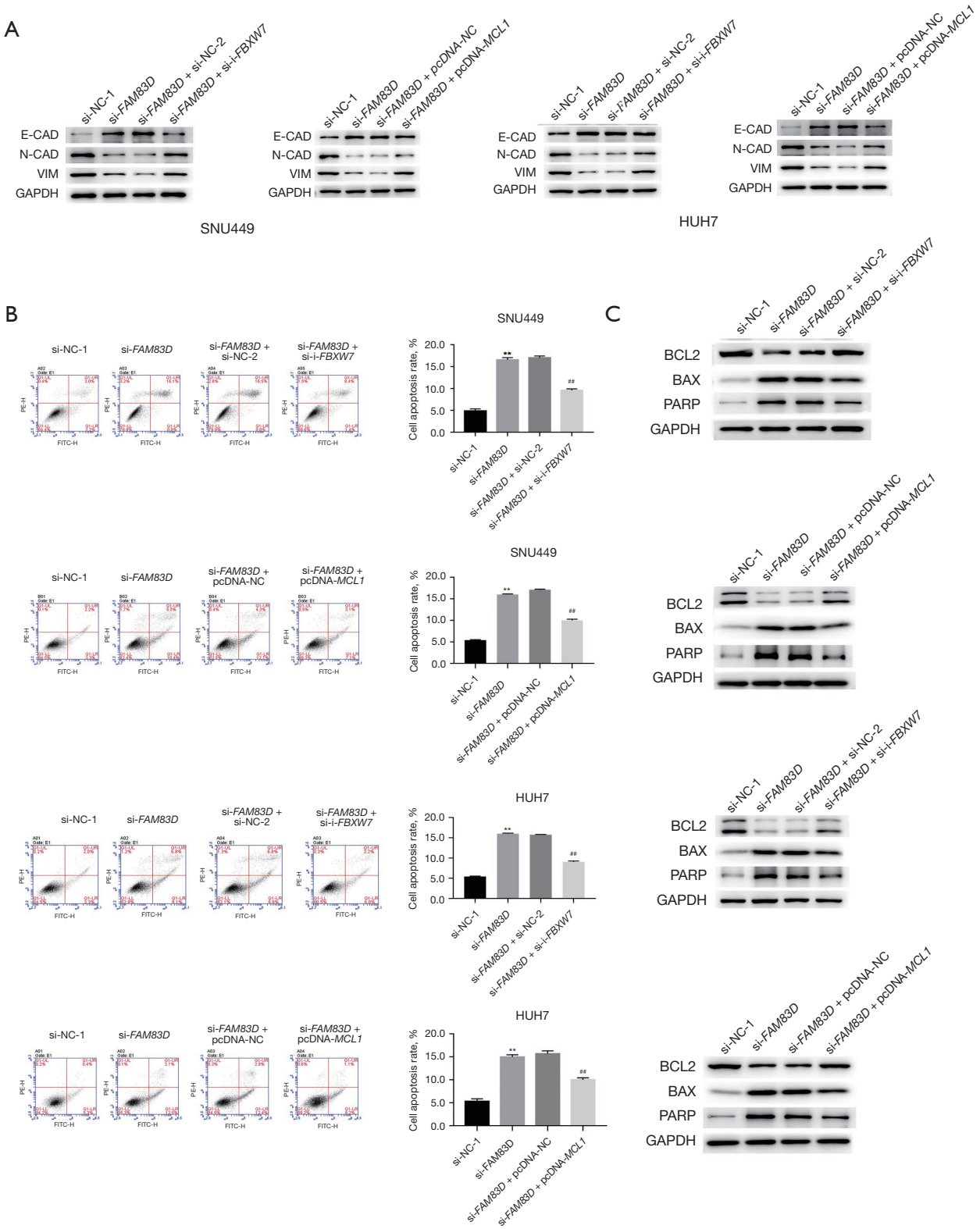

Figure 6 *FAM83D* activates *FBXW7*/*MCL1* pathway in HCC cells *in vitro* (HCC cells transfected with *FAM83D* siRNA; si*FAM83D* + si*FBXW7*; si*FAM83D* + pc*MCL1*). (A) The migration protein level was measured by western blot analysis. (B) Cell apoptosis was measured by flow cytometry. (C) The apoptotic-related protein level was measured by western blot analysis. **P<0.01; ##P<0.01.

FAM83D was closely related to a poor prognosis. Further, the results of the loss- and gain-of-function experiment *in vitro* showed that *FAM83D* promoted the proliferation and migration of HCC cells. The knockdown of *FAM83D* impaired tumor cell growth and migration, and promoted HCC cell apoptosis by increasing Bcl-2 and reducing Bax and PARP, while the upregulation of *FAM83D* reversed these results. Our findings provide novel insights into *FAM83D*-induced tumorigenesis and metastasis in HCC.

To achieve a deeper understanding of the role of *FAM83D* in the pathogenesis of HCC, we investigated the underlying pathways by which *FAM83D* affected HCC progression. F-box protein has received increasing attention due to its role in cancer. Among the F-box family, *FBXW7* is thought to be a tumor suppressor involved in cell proliferation and migration in cancers, including lung, breast, gastric, and pancreatic cancer (10,18).

A study has shown that hepatocyte-specific *FBXW7*-deficient mice exhibit hepatomegaly and steatohepatitis (19). Further, research has shown that the expression level of *FBXW7* affects the sensitivity of tumor cells to several chemotherapies (20,21). *MCL1* is a key pro-survival member of the BCL2 protein family. *MCL1* is one of the *FBXW7* substrates. *FBXW7* interacts with MCL-1 and degrades through phosphorylation (22). The loss of *FBXW7* leads to the accumulation of *MCL1*, which leads to the chemotherapy resistance of cancer cells (23). Notably, it has been confirmed that the low expression of *FBXW7* in squamous cell carcinoma increases the expression of MCL-1 and promotes resistance to standard chemotherapy (24). So we focused on the FBXW/*MCL1* signaling pathway. In our study, TCGA-LIHC analysis showed that the expression of *FAM83D* was negatively correlated with *FBXW7* expression, while *FBXW7* expression level was negatively correlated with *MCL1* expression level. we found that *FAM83D* enhanced the cell proliferation and migration and suppressed the cell apoptosis of the HCC cells by inhibiting the *FBXW7*/*MCL1* signaling pathway. After the knockdown of *FAM83D*, the expression of *FBXW7* protein was significantly increased and the expression of *MCL1* protein was reduced. Additionally, we found that *FBXW7* siRNA reversed the inhibitory effect of *FAM83D* knockdown on *MCL1* protein expression, which clearly indicated that the oncogenic function of *FAM83D* is at least.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2069/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2069/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2069/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and the relevant policies in China.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.

2. Wang W, Wei C. Advances in the early diagnosis of hepatocellular carcinoma. Genes Dis 2020;7:308-19.
3. Greten TF, Lai CW, Li G, et al. Targeted and Immune-Based Therapies for Hepatocellular Carcinoma. Gastroenterology 2019;156:510-24.
4. Meng T, Tong Z, Yang MY, et al. Immune implication of FAM83D gene in hepatocellular carcinoma. Bioengineered 2021;12:3578-92.
5. Shi R, Sun J, Sun Q, et al. Upregulation of FAM83D promotes malignant phenotypes of lung adenocarcinoma by regulating cell cycle. Am J Cancer Res 2016;6:2587-98.
6. Wang Z, Liu Y, Zhang P, et al. FAM83D promotes cell proliferation and motility by downregulating tumor suppressor gene FBXW7. Oncotarget 2013;4:2476-86.
7. Ishii N, Araki K, Yokobori T, et al. Reduced FBXW7 expression in pancreatic cancer correlates with poor prognosis and chemotherapeutic resistance via accumulation of MCL1. Oncotarget 2017;8:112636-46.
8. Hashimoto M, Kobayashi T, Tashiro H, et al. h-Prune is associated with poor prognosis and epithelial-mesenchymal transition in patients with colorectal liver metastases. Int J Cancer 2016;139:812-23.
9. Davis RJ, Welcker M, Clurman BE. Tumor suppression by the Fbw7 ubiquitin ligase: mechanisms and opportunities. Cancer Cell 2014;26:455-64.
10. Welcker M, Clurman BE. FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. Nat Rev Cancer 2008;8:83-93.
11. El-Mezayen H, Yamamura K, Yusa T, et al. MicroRNA-25 Exerts an Oncogenic Function by Regulating the Ubiquitin Ligase FBXW7 in Hepatocellular Carcinoma. Ann Surg Oncol 2021;28:7973-82.
12. Mu Y, Zou H, Chen B, et al. FAM83D knockdown regulates proliferation, migration and invasion of colorectal cancer through inhibiting FBXW7/Notch-1 signalling pathway. Biomed Pharmacother 2017;90:548-54.
13. Inuzuka H, Shaik S, Onoyama I, et al. SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. Nature 2011;471:104-9.
14. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013;41:D991-5.
15. Cancer Genome Atlas Research Network; Weinstein JN, Collisson EA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet 2013;45:1113-20.
16. Bozatzi P, Sapkota GP. The FAM83 family of proteins: from pseudo-PLDs to anchors for CK1 isoforms. Biochem Soc Trans 2018;46:761-71.
17. Hua YQ, Zhang K, Sheng J, et al. FAM83D promotes tumorigenesis and gemcitabine resistance of pancreatic adenocarcinoma through the Wnt/β-catenin pathway. Life Sci 2021;287:119205.
18. Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017;45:D777-83.
19. Onoyama I, Suzuki A, Matsumoto A, et al. FBXW7 regulates lipid metabolism and cell fate decisions in the mouse liver. J Clin Invest 2011;121:342-54.
20. Fang L, Yang Z, Zhou J, et al. Circadian Clock Gene CRY2 Degradation Is Involved in Chemoresistance of Colorectal Cancer. Mol Cancer Ther 2015;14:1476-87.
21. Gstalder C, Liu D, Miao D, et al. Inactivation of FBXW7 Impairs dsRNA Sensing and Confers Resistance to PD-1 Blockade. Cancer Discov 2020;10:1296-311.
22. Ren H, Koo J, Guan B, et al. The E3 ubiquitin ligases β-TrCP and FBXW7 cooperatively mediates GSK3-dependent Mcl-1 degradation induced by the Akt inhibitor API-1, resulting in apoptosis. Mol Cancer 2013;12:146.
23. Wertz IE, Kusam S, Lam C, et al. Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature 2011;471:110-4.
24. He L, Torres-Lockhart K, Forster N, et al. Mcl-1 and FBW7 control a dominant survival pathway underlying HDAC and Bcl-2 inhibitor synergy in squamous cell carcinoma. Cancer Discov 2013;3:324-37.

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