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Original Article

ATG10 overexpression is related to the dismal prognosis and promotes the growth and migration of hepatocellular carcinoma cells via cyclin B1/CDK1 and CDK2

Feng Li[1*], Kai Li[2*], Dan Li[3*], Wei Zhang[2], Kong-Wu Yang[1], Di Ke[1], Qiang Guo[4], Rong-Shu Shi[1]

[1]Department of Radiology, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China; [2]Department of Hepatobiliary and Pancreatic Surgery, The People's Hospital of Jianyang City, Jianyang, Sichuan, China; [3]Department of Oncology, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China; [4]Department of Cardiothoracic Surgery, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China. [*]Co-first authors.

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Abstract: Although the expression of autophagy-related 10 (ATG10) is known to be associated with the poor prognosis of cancer patients by enhancing cancer cell growth and migration, the roles of ATG10 in hepatocellular carcinoma (HCC) remains to be determined. In this study, the expression of ATG10 in HCC was analyzed using the data from TCGA databases and was further verified in the clinical samples from our patients. In addition, the relationships of ATG10 expression with clinical features, diagnosis and prognosis, as well as the predictive values of ATG10 expression in overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI) were explored. Furthermore, the expression and the prognostic values of ATG10 co-expressed genes were also identified in HCC, which was used to construct prognostic nomograms. Our data showed that the expression level of ATG10 was significantly increased in HCC, and the elevated ATG10 expression was associated with poor prognosis. Moreover, cells with ATG10 knockdown were used to investigate the effects of ATG10 on HCC cell proliferation and migration. We found that silencing ATG10 inhibited the proliferation, migration, and invasion of HCC cells, which was related to the protein expression of cyclin B1, CDK1, and CDK2. Similarly, the overexpression of ATG10 co-expressed genes ATG12, LARS1, CWC27, and SLC30A5 in HCC patients were also associated with the OS, DSS, and PFI. The risk models and nomograms based on ATG10 and ATG10 co-expressed genes indicated the correclation between their expression and the dismal prognosis in HCC patients. In conclusion, ATG10 expression was elevated in HCC and was associated with poor prognosis. Inhibition of ATG10 expression could attenuate cancer progression. ATG10-related nomograms and risk models could be used clinically to evaluate the prognosis of HCC patients.

Keywords: ATG10, nomogram, risk model, prognosis, hepatocellular carcinoma

Introduction

Liver cancer is a common malignant tumor globally, ranking the seventh in the incidence of cancer patients and the second in cancer-related mortality [1, 2]. Given the lack of specific clinical symptoms in the early stages of liver cancer, patients often have advanced stage disease at the time of diagnosis. Although there have been significant improvements in surgery, radiation therapy, chemotherapy and other treatment strategies resulting in improved 5-year survival during the past decade, the prognosis of patients with liver cancer remains poor. Moreover, the 5-year recurrence rate is as high as 70% [3, 4]. Therefore, early diagnosis is crucial to prolong the overall survival (OS) and provide optimal clinical treatment of patients with hepatocellular carcinoma (HCC).

In recent years, targeted therapy has become the new treatment modality to improve the prognosis of cancer patients, and many genes have been identified to be associated with liver cancer progression [5-8]. For example, the expression level of Schlafen family member 11

(SLFN11) is significantly decreased in HCC and is associated with short OS and a high recurrence rate. In line with this finding, overexpression of SLFN11 inhibits cell proliferation, migration and invasion, as well as promotes apoptosis, while inhibition of SLFN11 expression shows the opposite effects. Mechanistically, SLFN11 combined with ribosomal protein S4 X-linked (RPS4X) blocks the mTOR signaling pathway and reverses HCC progression, acting as a tumor suppressor [5]. On the other hand, ubiquitin-conjugating enzyme E2T (UBE2T) expression is upregulated in HCC tissues and cells. High UBE2T expression is associated with a poor prognosis in HCC patients. Consistently, UBE2T overexpression enhances HCC cell proliferation and migration. Notably, miR-212-5p targets and regulates UBE2T expression, thereby enhancing the malignant phenotype of HCC cells [6]. Besides, constitutive photomorphogenesis 9 (COP9) signalosome subunit 6 (CSN6) has been reported to be significantly upregulated in HCC tissues. CSN6 overexpression is associated with the dismal prognosis of HCC patients. Similarly, CSN6 overexpression promotes, while CSN6 silencing inhibits, HCC cell proliferation, migration and invasion. Mechanistic studies have revealed that CSN6 promotes epithelial-mesenchymal transition (EMT) by inhibiting the E-cadherin expression, activating the MEK/ERK signaling pathway, and upregulating snail expression [8].

Autophagy-related 10 (ATG10), also known as APG10 and APG10L, is an E2-like enzyme involved in ubiquitin-like modifications necessary for autophagosome formation. Accumulating evidence has substantiated that ATG10 plays an important role in the carcinogenesis of lung, colorectal, and gastric cancers [9-14]. ATG10 overexpression has been reported in non-small cell lung cancer (NSCLC) and can predict patient survival. Indeed, ATG10 can promote NSCLC cell proliferation and migration [9]. In addition, ATG10 upregulation has been reported in colorectal cancer (CRC), which is closely related to tumor lymph node metastasis and invasion. These findings suggest that ATG10 may be a potential prognostic marker for CRC patients [11]. Furthermore, the expression level of miR-27b-3p is found to be significantly downregulated in oxaliplatin-resistant cells and is associated with disease-free survival in CRC patients. Current evidence suggests that miR-27b-3p sensitizes CRC cells to oxaliplatin in vitro and in vivo and can induce autophagy by inhibiting ATG10 expression [12]. Another ATG10 regulation has been reported in the study with podofilox treatment which induces cell cycle arrest and is involved in c-Myc and p53 gene expression. Podofilox is found to inhibit the expression of ATG10 in gastric cancer tissues, thereby attenuating the proliferation and colony formation of gastric cancer cells [14]. However, the roles and mechanisms of ATG10 in HCC have not been reported. Therefore, in this study, we investigated the roles of ATG10 via in silico analysis and experimental approaches to identify portential targets for the treatment of HCC patients.

Materials and methods

ATG10 expression in HCC tissues

The gene expression data of ATG10 in 374 cancer tissues and 50 adjacent normal tissues were obtained from the TCGA database. In addition, the differential expression of ATG10 in 50 paired HCC and normal tissues from the the TCGA database was analyzed. Furthermore, the expression of ATG10 in the tissue samples of 8 HCC patients in our hospital were examined. all patients have signed informed consent. This study was approved by the Ethics Committee of our hospital (KLL-2022-703).

The relationship between ATG10 expression and the diagnosis as well as the prognosis of HCC

The diagnostic value of ATG10 expression level in HCC was determined by receiver operating characteristic (ROC) analysis using the data from TCGA database, and the relationship between the elevated ATG10 expression level and the OS, disease-specific survival (DSS) and progression-free interval (PFI) of HCC patients was identified by Kaplan-Meier (K-M) survival analysis. In addition, the relationship between ATG10 expression level and the prognosis of HCC patients was investigated in HCC patient's clinicopathological features such as T stage, N stage, M stage, pathological stage, age, gender.

Construction of nomograms

Univariate Cox regression analysis was performed to determine the relationship of ATG10 expression level with N stage, M stage, T stage, pathological stage, tumor status, gender, age, alpha-fetoprotein (AFP), Fibrosis ishak score (FIS), and the prognosis of HCC patients. Statistically significant clinicopathological features ($P<0.05$) were incorporated into multivariate Cox regression analysis. The prognostic nomograms of HCC patients were constructed based on the multivariate Cox regression analysis results.

Cell culture and model construction of ATG10

Normal human liver LO2 cells and HCC cells (LM3, Hep3B, PLC, HepG2, and Huh7) were cultured in MEM or DMEM medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and maintained in 5% CO_2 incubator (Thermo, Germany) at 37°C. For cell transfection, Hep3B and PLC cells were seeded into 6-well plates and transfected with indicated siRNA using the Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's instructions [9]. The cells were harvested at 24 h after transfection, and the transfection efficiency was verified by quantitative reverse transcription PCR (qRT-PCR) and western blotting. The siRNA of ATG10 and the negative control siRNA were purchased from the GemmaGene (Shanghai, China). The siRNA sequence was as follows: sense 5'-GAGUUCAUGAGUGCUAATT-3', antisense 5'-UUAUAGCACU CA UGAACUCTT-3'.

qRT-PCR

Total RNAs were extracted from cells and tissues using TRIZOL reagent. Reverse transcription was performed according to standard protocols to produce cDNA as template for PCR amplification using GAPDH as the internal reference [15]. The PCR primers for ATG10 were: forward 5-GGTGATAGTTGGGAATGGAGACC-3 and reverse 5-GTCTGTCCATGGGTAGATGCTC-3.

Western blot analysis

Briefly, the total protein of transfected Hep3B and PLC cells was extracted by RIPA cell lysis buffer containing protease inhibitors and quantified by BCA protein detection kit (Beyotime, China). Then, the proteins were separated by 10% SDS-PAGE (Solarbio, China) and transferred onto polyvinylidene fluoride (PVDF) membrane. The membrane was blocked by 5% skimmed milk in TBST for 1 h at room temperature and incubated with primary antibodies overnight at 4°C. The primary antibodies used in this study were: ATG10 (1:1000, BOSTER, Wuhan, China), Cyclin B1 (1:1000, BOSTER, Wuhan, China), cyclin-dependent kinase (CDK) 1 (1:1000, BOSTER, Wuhan, China), CDK2 (1:1000, BOSTER, Wuhan, China), as well as β-actin (1:3000, BOSTER, Wuhan, China). After extensive washing, the membrane was incubated with the corresponding secondary antibody for 1 h at room temperature. The protein expression was developed by a chemiluminescence detection kit, and the intensity of the signal was analyzed with Image J.

CCK-8 assay

The effects of ATG10 on HCC cell proliferation were detected by the CCK-8 assay according to the manufacturer's instruction. Briefly, HCC cells transfected with control siRNA or siATG10 were plated into 96-well plates at 3000 cells/well. Then, 10 ul of CCK-8 solution was added to each well at 0, 24, 48, 72, and 96 h time points and incubated for 2 h, and the optical density (OD) values at 450 nm were detected with a spectrophotometer.

Cell invasion and migration assay

Transwell was used for cell migration and invasion assay. For invasion assay, the membrane in the upper chamber of Transwell was coated with Matrigel. Briefly, about 1×10^5 Hep3B and PLC cells in serum free medium were seeded into the upper chamber of Tranwell, while complete medium with 20% fetal bovine serum was added to the lower chamber of Transwell. After overnight culture, the non-invasive cells in the upper chamber were removed by gently swabbing with cotton-tipped applicators, and the cells on the lower side of the membrane were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet solution for 2 h. The cells were accounted under microscope and analyzed using the Image J software.

Analysis of ATG10 co-expressed genes

The ATG10 co-expressed genes were filtered using correlation analysis with the screening criteria: P<0.001 and an absolute value of the correlation coefficient >0.4. The genes related to the OS, DSS, and PFI in HCC patients were obtained by Cox regression analysis in Xiantao Academic Website, and the significant prognosis-related genes were identified based on the screening criteria P<0.01. Overlapped genes between the prognosis-related genes and ATG10 co-expressed genes were identified by a Venn plot.

Construction and identification of ATG10-associated nomograms and risk models

The expression levels of ATG10 co-expressed genes ATG12, LARS1, CWC27 and SLC30A5 were identified in normal tissues and HCC tissues, and the relationship between the expression levels of ATG12, LARS1, CWC27 and SLC30A5, and OS, DSS and PFI in HCC patients was visualized by K-M survival analysis. Subsequently, the nomograms related to the expression of ATG10, ATG12, LARS1, CWC27 and SLC30A5 were constructed. The least absolute shrinkage and selection operator (LASSO) regression analysis was used to explore the relationship between the expression of ATG10, ATG12, LARS1, CWC27 and SLC30A5 and the prognosis of HCC patients to obtain the variable coefficient, which was used to calculate the risk score of HCC tissues and to generate a variable coefficient trajectory map. The differences in survival between the high- and the low-risk groups were investigated by K-M survival analysis.

Statistical analysis

The gene expression in HCC tissues and the statistical results in cell model were analyzed by the Wilcoxon rank sum test or t test. Survival analysis, Cox regression analysis, and LASSO analysis were performed to identify the OS, PFI, and DSS-related genes in HCC patients. Correlation analysis was used to screen ATG10 co-expressed genes. A *P*-value <0.05 was considered statistically significant.

Results

ATG10 overexpression was significantly associated with the diagnosis and the poor prognosis of HCC patients

First, we found that ATG10 expression was significantly upregulated in the tumor samples of both paired and unmatched HCC patients (**Figure 1A** and **1B**). ROC analysis showed that the area under the curve of ATG10 expression level in HCC tissues and normal tissues was 0.927 (**Figure 1C**), suggesting that ATG10 overexpression level had a significant diagnostic value in HCC. Furthermore, K-M survival analysis revealed that the elevated ATG10 expression was associated with the dismal prognosis of HCC patients (**Figure 1D-F**) with HR values of 2.14, 2.05 and 1.48 for OS, DSS, and PFI, respectively.

ATG10 overexpression was significantly associated with the OS, DSS and PFI of HCC patients in subgroup analysis

As shown in **Figures 2** and S1, the elevated ATG10 expression was significantly associated with the OS of HCC patients with the following clinicopathological characteristics: T1, T1-2, T1-3, T3-4, N0, M0, pathological stage (I, I-II, I-III and III), Tumor status, male, female, age (>60 year), weight (≤70 kg), weight (>70 kg), BMI (≤25), BMI (>25), R0 resection, G1-3, G2-3, G2-4, G3, G3-4, AFP (>400 ng/ml), no vascular invasion, and height (<170 cm). In addition, the elevated ATG10 expression was significantly associated with the DSS of HCC patients with the following clinicopathological characteristics: T1, T1-2, T1-3, N0, M0, pathological stage (I, I-II and I-III), tumor status, male, age (>60 years), weight (>70 kg), BMI (>25), R0 resection, G1-3, G2-3, G3-4, AFP (≤400 ng/ml), AFP (>400 ng/ml), no vascular invasion, and height (<170 cm) (**Figures 3** and S2). On the other hand, the elevated ATG10 expression was significantly associated with the PFI of HCC patients with the following clinicopathological characteristics: T1-2, T1-3, N0, M0, pathological stage (I-II and I-III), male, female, age (>60 years), weight (≤70 kg), BMI (≤25), G1-3, G2-4, G3-4, AFP (>400 ng/ml), vascular invasion, and height (<170 cm) (**Figure 4**).

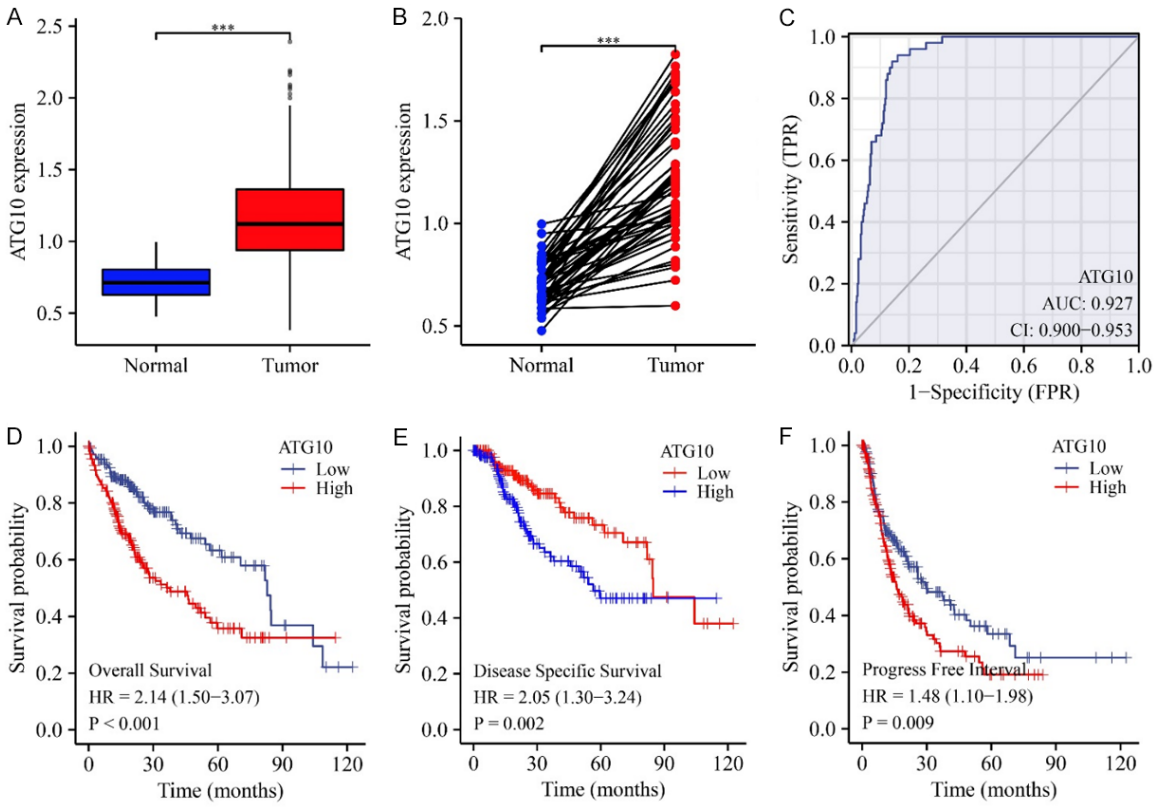


Figure 1. The expression levels and clinical features of ATG10 in HCC. A, B. The expression levels of ATG10 in HCC; C. Diagnostic value using ROC analysis; D. OS; E. DSS; F. PFI. Note: HCC, hepatocellular carcinoma; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; ROC, receiver operating characteristic.

ATG10-related prognostic nomograms

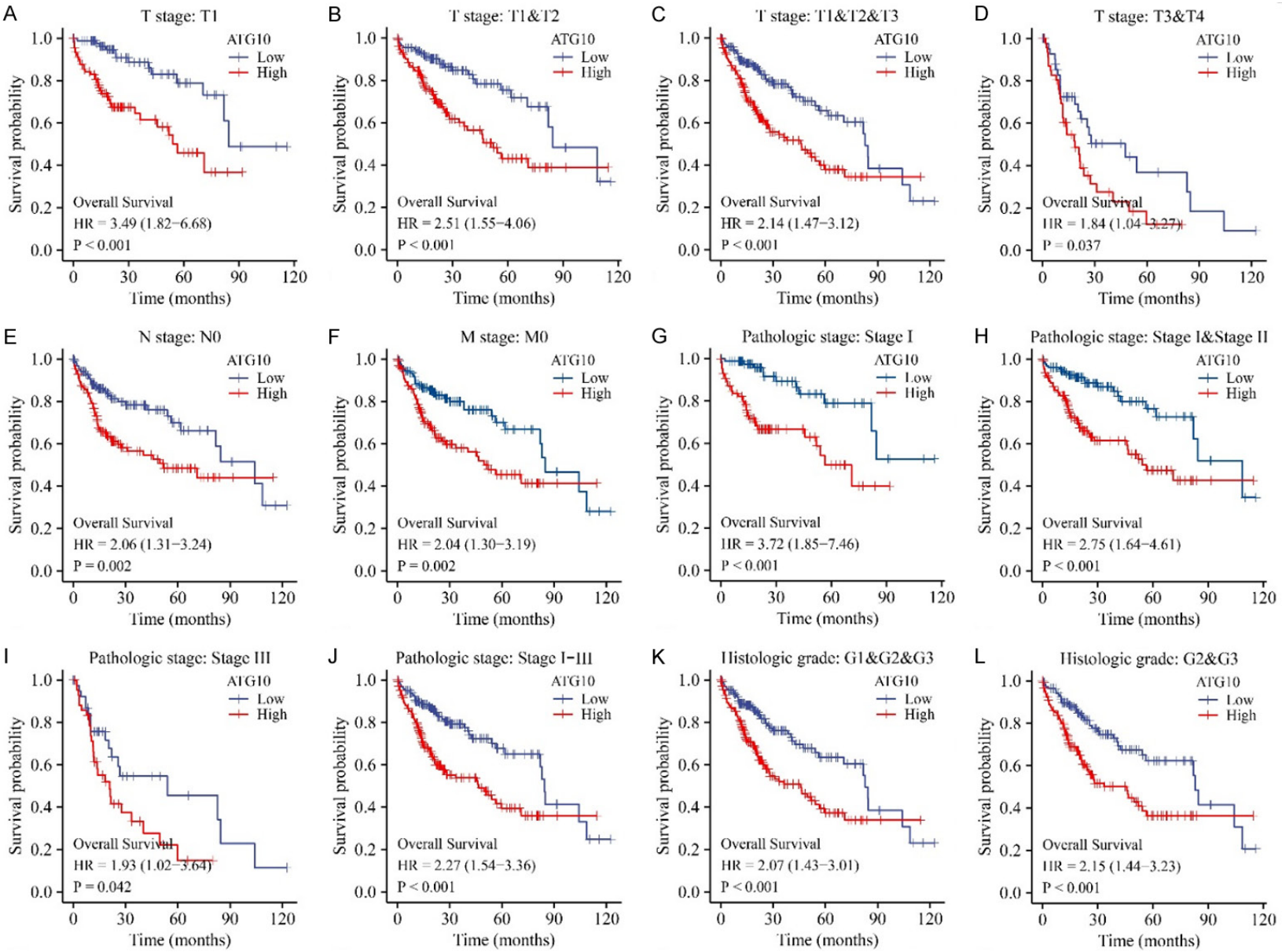
Using Cox regression analysis, we found that the tumor status and the elevated ATG10 expression level could independently predict the OS of HCC patients (**Table 1**). In addition, the elevated ATG10 expression could independently predict the DSS of HCC patients (**Table 2**). Notably, the tumor status could independently predict the PFI in HCC patients (**Table 3**). Therefore, based on the Cox regression analysis results, we constructed nomograms using tumor status and ATG10 expression to predict the OS, DSS, and PFI in HCC patients (**Figures 5** and S3).

Inhibition of ATG10 expression could delay the growth and the mobility of HCC cells via cyclin B1/CDK1 and CDK2 signaling pathways

Importantly, the analysis of HCC samples from our hospital showed that ATG10 mRNA expression levels were upregulated in tumor samples, consistent with the results from bioinformatics analysis (**Figure 6A**). Moreover, we experimentally examined the function of ATG10 on the malignant phenotype of HCC cells by silencing the expression of ATG10 with siRNA in several HCC cell lines (**Figure 6B-D**). The results showed that ATG10 silencing not only significantly suppressed the proliferation of HCC cells, as determined by CCK-8 assay, which was related to the expression levels of cyclin B1, CDK1, and CDK2 (**Figure 6E-G**), but also inhibited the migration and invasion of HCC cells (**Figure 7A-D**).

Overexpression of ATG10 co-expressed genes was significantly associated with the prognosis of HCC patients

We also explored the significant factors that were associated with the OS, PFI and DSS of HCC patients by performing Cox regression analysis with a *P*-value <0.01 as the screening criteria via Xiantao Academic Website. The prognosis-related genes and 55 ATG10 co-expressed genes were intersected to obtain


A
T stage: T1
ATG10
Low
High
Survival probability
Overall Survival
HR = 3.49 (1.82−6.68)
P < 0.001
Time (months)
B
T stage: T1&T2
ATG10
Low
High
Survival probability
Overall Survival
HR = 2.51 (1.55−4.06)
P < 0.001
Time (months)
C
T stage: T1&T2&T3
ATG10
Low
High
Survival probability
Overall Survival
HR = 2.14 (1.47−3.12)
P < 0.001
Time (months)
D
T stage: T3&T4
ATG10
Low
High
Survival probability
Overall Survival
HR = 1.84 (1.04−3.27)
P = 0.037
Time (months)
E
N stage: N0
ATG10
Low
High
Survival probability
Overall Survival
HR = 2.06 (1.31−3.24)
P = 0.002
Time (months)
F
M stage: M0
ATG10
Low
High
Survival probability
Overall Survival
HR = 2.04 (1.30−3.19)
P = 0.002
Time (months)
G
Pathologic stage: Stage I
ATG10
Low
High
Survival probability
Overall Survival
HR = 3.72 (1.85−7.46)
P < 0.001
Time (months)
H
Pathologic stage: Stage I&Stage II
ATG10
Low
High
Survival probability
Overall Survival
HR = 2.75 (1.64−4.61)
P < 0.001
Time (months)
I
Pathologic stage: Stage III
ATG10
Low
High
Survival probability
Overall Survival
HR = 1.93 (1.02−3.64)
P = 0.042
Time (months)
J
Pathologic stage: Stage I−III
ATG10
Low
High
Survival probability
Overall Survival
HR = 2.27 (1.54−3.36)
P < 0.001
Time (months)
K
Histologic grade: G1&G2&G3
ATG10
Low
High
Survival probability
Overall Survival
HR = 2.07 (1.43−3.01)
P < 0.001
Time (months)
L
Histologic grade: G2&G3
ATG10
Low
High
Survival probability
Overall Survival
HR = 2.15 (1.44−3.23)
P < 0.001
Time (months)

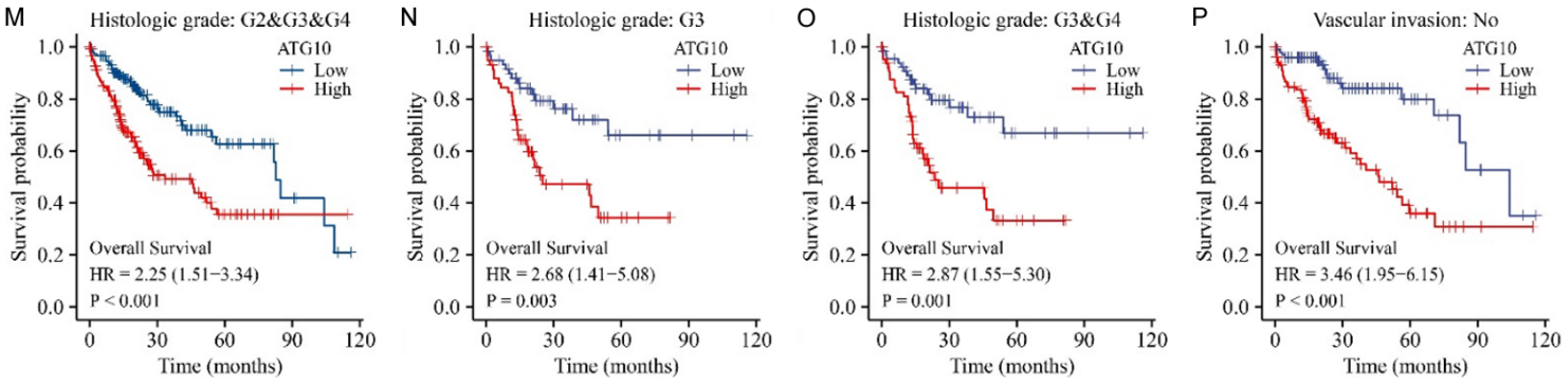
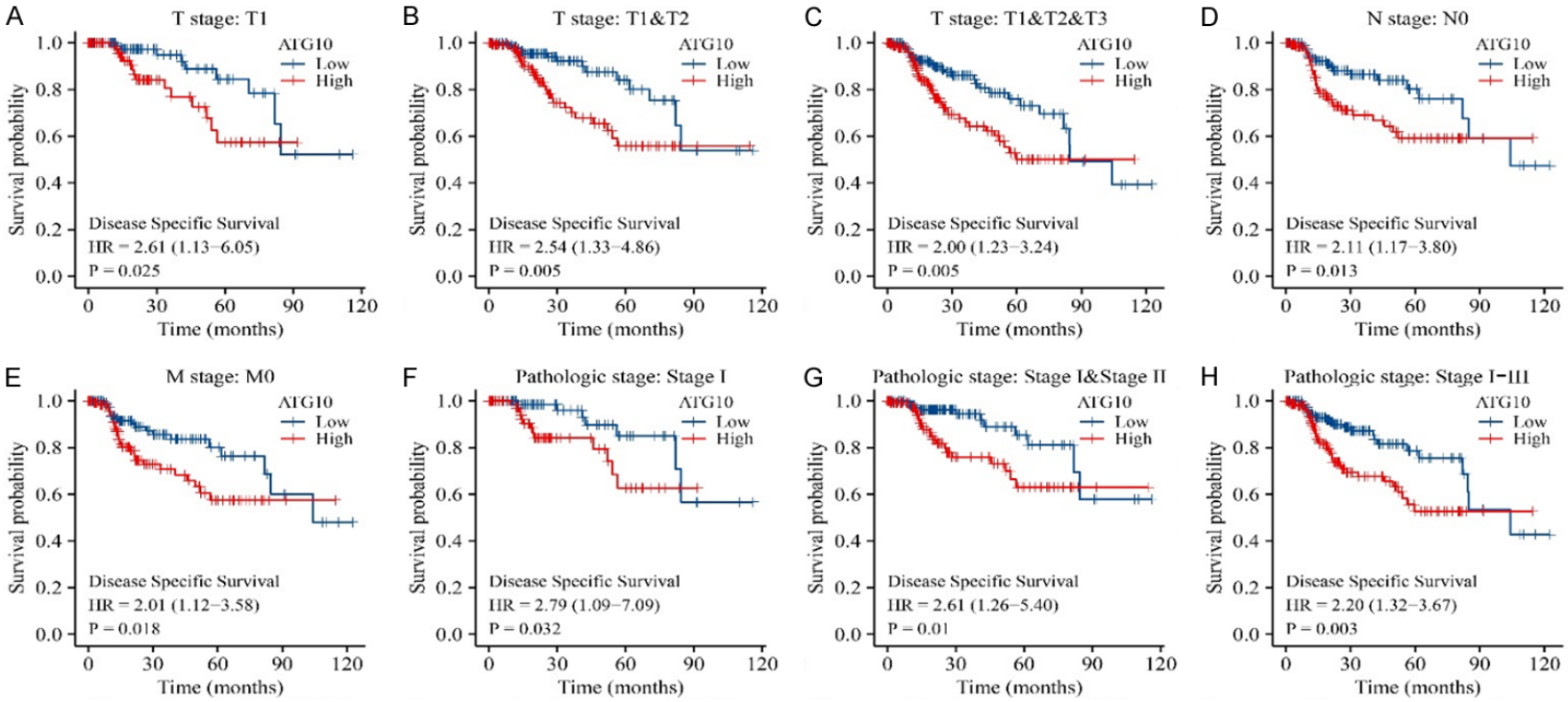


Figure 2. The relationship between ATG10 overexpression and the OS determined by subgroup analysis in HCC. A. T1; B. T1-2; C. T1-3; D. T3-4; E. N0; F. M0; G. Stage I; H. Stage I-II; I. Stage III; J. Stage I-III; K. G1-3; L. G2-3; M. G2-4; N. G3; O. G3-4; P. No vascular invasion. Note: HCC, hepatocellular carcinoma; OS, overall survival.



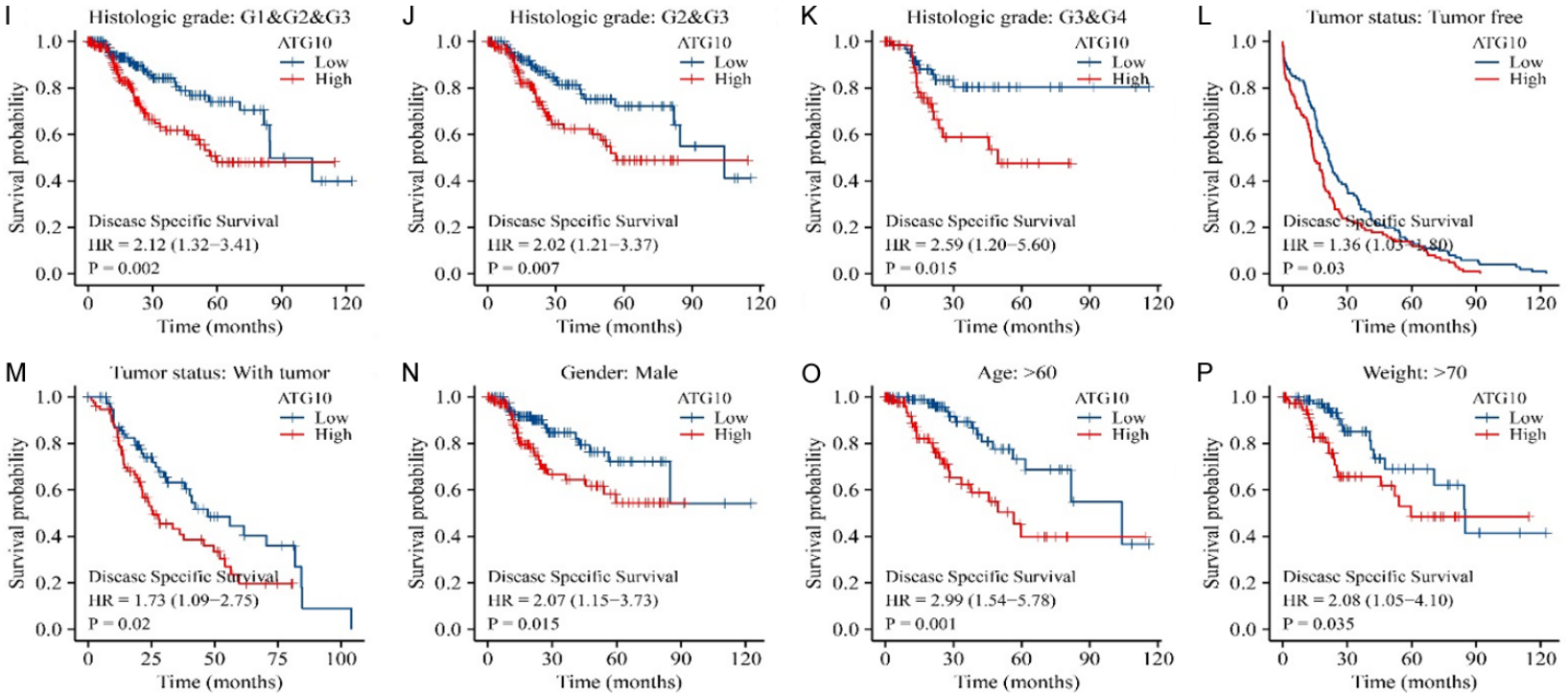
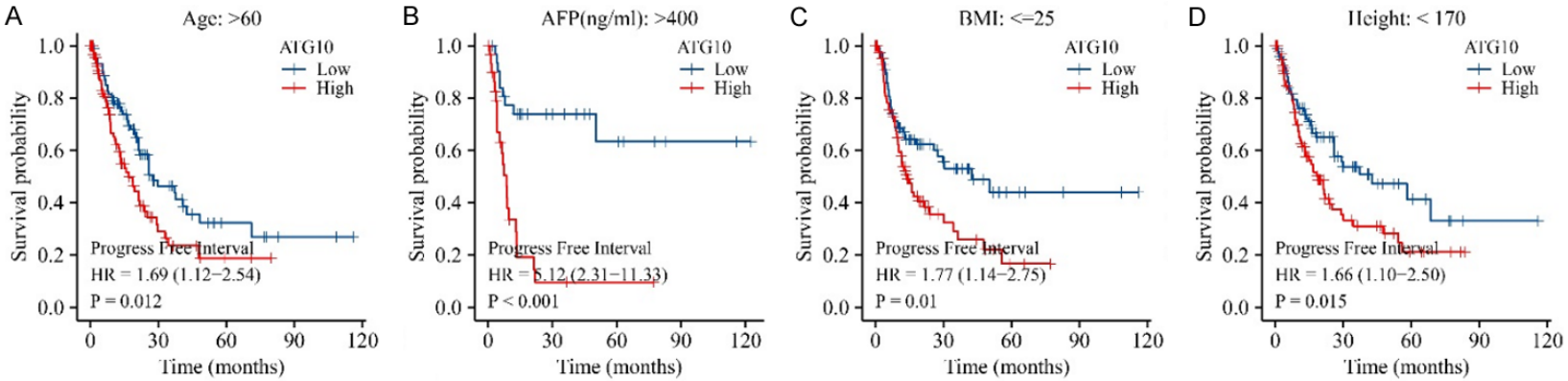


Figure 3. The relationship between ATG10 overexpression and the DSS determined by subgroup analysis. A. T1; B. T1-2; C. T1-3; D. N0; E. M0; F. Stage I; G. Stage I-II; H. Stage I-III; I. G1-3; J. G2-3; K. G3-4; L. Tumor free; M. Tumor; N. Male; O. Age; P. Weight. Note: HCC, hepatocellular carcinoma; DSS, disease-specific survival.



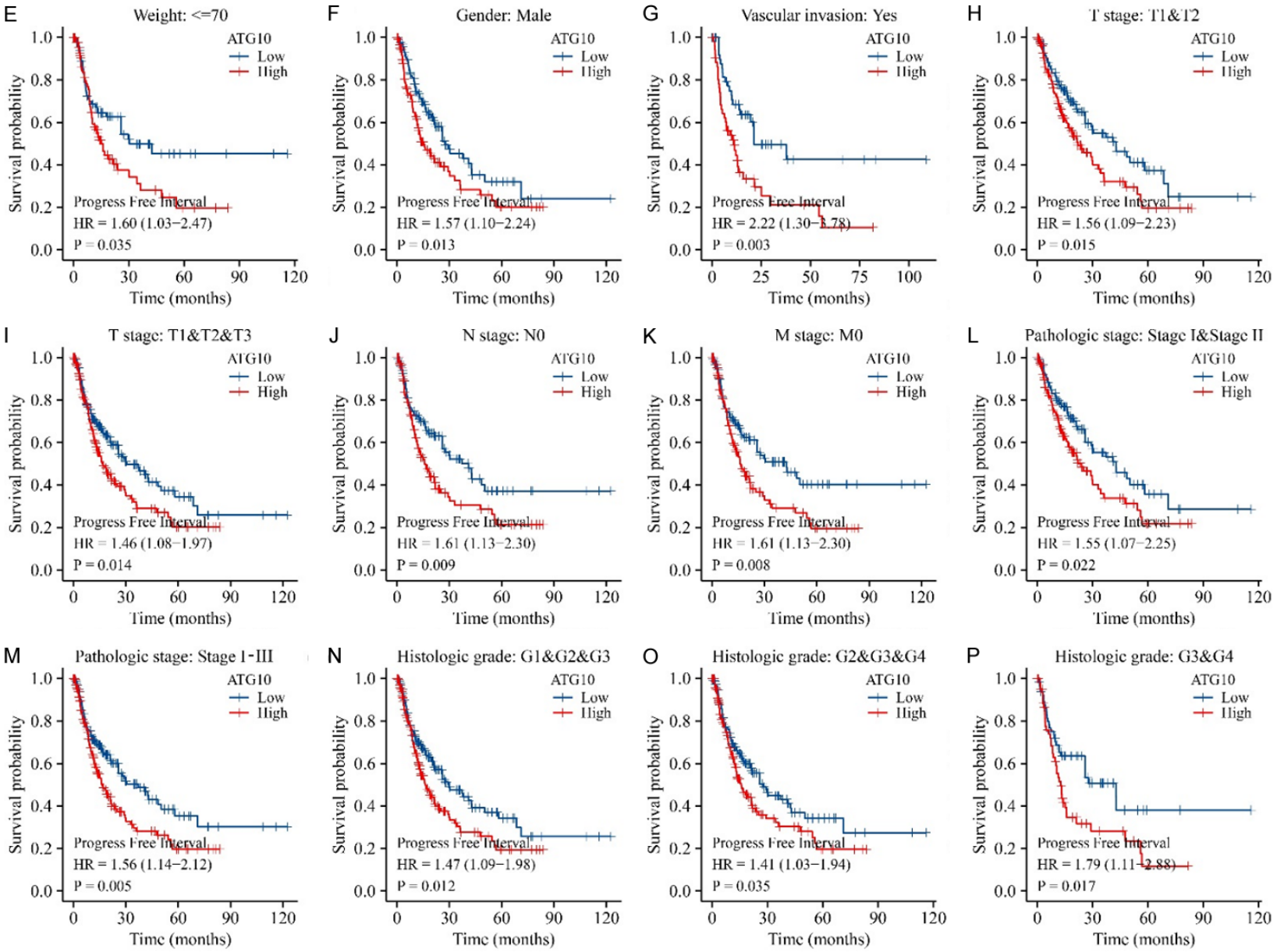

E
Weight: <=70
ATG10
Low
High
Survival probability
Progress Free Interval
HR = 1.60 (1.03−2.47)
P = 0.035
Time (months)
F
Gender: Male
HR = 1.57 (1.10−2.24)
P = 0.013
G
Vascular invasion: Yes
HR = 2.22 (1.30−3.78)
P = 0.003
H
T stage: T1&T2
HR = 1.56 (1.09−2.23)
P = 0.015
I
T stage: T1&T2&T3
HR = 1.46 (1.08−1.97)
P = 0.014
J
N stage: N0
HR = 1.61 (1.13−2.30)
P = 0.009
K
M stage: M0
HR = 1.61 (1.13−2.30)
P = 0.008
L
Pathologic stage: Stage I&Stage II
HR = 1.55 (1.07−2.25)
P = 0.022
M
Pathologic stage: Stage I-III
HR = 1.56 (1.14−2.12)
P = 0.005
N
Histologic grade: G1&G2&G3
HR = 1.47 (1.09−1.98)
P = 0.012
O
Histologic grade: G2&G3&G4
HR = 1.41 (1.03−1.94)
P = 0.035
P
Histologic grade: G3&G4
HR = 1.79 (1.11−2.88)
P = 0.017

Figure 4. The relationship between ATG10 overexpression and the PFI determined by subgroup analysis in HCC. A. Age; B. AFP; C. BMI; D. Height; E. Weight; F. Male; G. Vascular invasion; H. T1-2; I. T1-3; J. N0; K. M0; L. Stage I-II; M. Stage I-III; N. G1-3; O. G2-4; P. G3-4. Note: HCC, hepatocellular carcinoma; PFI, progression-free interval.

Table 1. Risk factors for the OS of HCC patients

Characteristics	N	HR (95% CI)	P	HR (95% CI)	P
N stage	258				
N0	254	Reference			
N1	4	2.029 (0.497-8.281)	0.324		
M stage	272				
M0	268	Reference			
M1	4	4.077 (1.281-12.973)	0.017	1.857 (0.135-25.536)	0.644
T stage	370				
T1	183	Reference			
T2	94	1.431 (0.902-2.268)	0.128	0.000 (0.000-Inf)	0.995
T3	80	2.674 (1.761-4.060)	<0.001	1.233 (0.164-9.240)	0.839
T4	13	5.386 (2.690-10.784)	<0.001	1.954 (0.225-16.928)	0.543
Pathologic stage	349				
Stage I	173	Reference			
Stage II	86	1.417 (0.868-2.312)	0.164	4077070.461 (0.000-Inf)	0.995
Stage III	85	2.734 (1.792-4.172)	<0.001	2.322 (0.305-17.691)	0.416
Stage IV	5	5.597 (1.726-18.148)	0.004		
Tumor status	354				
Tumor free	202	Reference			
With tumor	152	2.317 (1.590-3.376)	<0.001	1.729 (1.076-2.778)	0.024
Gender	373				
Female	121	Reference			
Male	252	0.793 (0.557-1.130)	0.200		
Age	373				
≤60	177	Reference			
>60	196	1.205 (0.850-1.708)	0.295		
AFP (ng/ml)	279				
≤400	215	Reference			
>400	64	1.075 (0.658-1.759)	0.772		
FIS	214				
0	75	Reference			
1/2	31	0.935 (0.437-2.002)	0.864		
3/4	28	0.698 (0.288-1.695)	0.428		
5/6	80	0.737 (0.410-1.325)	0.308		
ATG10	373				
Low	187	Reference			
High	186	2.144 (1.496-3.072)	<0.001	2.087 (1.282-3.398)	0.003

Note: FIS, Fibrosis Ishak score; OS, overall survival; HCC, hepatocellular carcinoma; HR, HR, hazard ratio; CI, confidence interval.

genes associated with the prognosis of HCC. We revealed that the overlapping genes ATG12, LARS1, CWC27, and SLC30A5 were overexpressed in unpaired and paired tissues of HCC patients (**Figure 8**). Furthermore, the upregulation of ATG12, LARS1, CWC27, and SLC30A5 was significantly associated with shorter OS, DSS, and PFI of HCC patients (**Figure 9**). As

Table 2. Risk factors for the DSS of HCC patients

Characteristics	N	HR (95% CI)	P	HR (95% CI)	P
N stage	253				
N0	249	Reference			
N1	4	3.612 (0.870-14.991)	0.077		
M stage	268				
M0	265	Reference			
M1	3	5.166 (1.246-21.430)	0.024	5.318 (0.344-82.166)	0.232
T stage	362				
T1	180	Reference			
T2	92	1.625 (0.872-3.028)	0.126	0.000 (0.000-Inf)	0.997
T3	77	3.748 (2.177-6.451)	<0.001	0.870 (0.114-6.656)	0.894
T4	13	10.164 (4.480-23.060)	<0.001	1.568 (0.160-15.385)	0.700
Pathologic stage	341				
Stage I	170	Reference			
Stage II	84	1.561 (0.776-3.141)	0.212	17530559.455 (0.000-Inf)	0.997
Stage III	83	4.288 (2.438-7.543)	<0.001	5.087 (0.651-39.756)	0.121
Stage IV	4	9.369 (2.171-40.437)	0.003		
Tumor status	354				
Tumor free	202	Reference			
With tumor	152	775790759.389 (0.000-Inf)	0.994		
Gender	365				
Female	118	Reference			
Male	247	0.813 (0.516-1.281)	0.373		
Age	365				
≤60	174	Reference			
>60	191	0.846 (0.543-1.317)	0.458		
AFP (ng/ml)	275				
≤400	214	Reference			
>400	61	0.867 (0.450-1.668)	0.668		
FIS	210				
0	73	Reference			
1/2	31	1.523 (0.639-3.630)	0.342		
3/4	28	0.632 (0.183-2.191)	0.470		
5/6	78	0.788 (0.363-1.708)	0.545		
ATG10	365				
Low	184	Reference			
High	181	2.055 (1.302-3.244)	0.002	2.089 (1.143-3.818)	0.017

Note: FIS, Fibrosis Ishak score; DSS, disease-specific survival; HCC, hepatocellular carcinoma; HR, HR, hazard ratio; CI, confidence interval.

shown in **Figures 10** and S4, nomograms constructed based on the expression of ATG10, ATG12, LARS1, CWC27, and SLC30A5 could predict the OS, DSS, and PFI in HCC patients. To confirm these findings, we also built several risk models based on the expression of ATG10 co-expressed genes. As expected, LASSO regression analysis in the risk model built based on the expression of ATG10, LARS1 and CWC27 also showed that HCC patients in the high-risk group had a poorer OS (**Figure 11A**). Similarly, in the risk model that was constructed based on the expression levels of ATG10, ATG12, and CWC27, HCC patients in the high-risk group had worse DSS (**Figure 11B**). Consistently, in the risk model that was constructed based on the expression of ATG10, ATG12, CWC27, and SLC30A5, HCC patients in

Table 3. Risk factors for the PFI of HCC patients

Characteristics	N	HR (95% CI)	P	HR (95% CI)	P
N stage	258				
N0	254	Reference			
N1	4	1.370 (0.338-5.552)	0.659		
M stage	272				
M0	268	Reference			
M1	4	3.476 (1.091-11.076)	0.035	2.649 (0.222-31.669)	0.442
T stage	370				
T1	183	Reference			
T2	94	2.020 (1.411-2.892)	<0.001	1.643 (0.090-29.890)	0.737
T3	80	2.620 (1.811-3.789)	<0.001	1.137 (0.154-8.394)	0.900
T4	13	4.266 (2.181-8.347)	<0.001	0.946 (0.109-8.238)	0.960
Pathologic stage	349				
Stage I	173	Reference			
Stage II	86	1.905 (1.305-2.780)	<0.001	0.652 (0.034-12.450)	0.776
Stage III	85	2.678 (1.857-3.861)	<0.001	1.633 (0.220-12.107)	0.631
Stage IV	5	5.576 (1.728-17.992)	0.004		
Tumor status	354				
Tumor free	202	Reference			
With tumor	152	11.342 (7.567-17.000)	<0.001	15.191 (9.046-25.510)	<0.001
Gender	373				
Female	121	Reference			
Male	252	0.982 (0.721-1.338)	0.909		
Age	373				
≤60	177	Reference			
>60	196	0.960 (0.718-1.284)	0.783		
AFP (ng/ml)	279				
≤400	215	Reference			
>400	64	1.045 (0.698-1.563)	0.832		
FIS	214				
0	75	Reference			
1/2	31	1.420 (0.799-2.524)	0.232		
3/4	28	1.353 (0.746-2.451)	0.319		
5/6	80	1.345 (0.861-2.101)	0.193		
ATG10	373				
Low	187	Reference			
High	186	1.477 (1.103-1.977)	0.009	1.051 (0.725-1.522)	0.794

Note: FIS, Fibrosis Ishak score; HCC, hepatocellular carcinoma; PFI, progression-free interval; HR, hazard ratio; CI, confidence interval.

the high-risk group had a worse PFI (**Figure 11C**).

Discussion

Multiple lines of evidence suggests that changes in the expression levels of some genes are associated with cancer progression, which can predict the prognosis of cancer patients [7, 16-18]. For example, the expression of mitochondrial fission regulator 2 (MTFR2) was reported to be upregulated in HCC tissues and correlated with the OS, indicating that the elevated expression of MTFR2 was an independent risk factor for the dismal prognosis of HCC patients [7]. In addition, Rho GTPase activating protein 9 (ARHGAP9) expression was found to be decreased in HCC tissues and associated

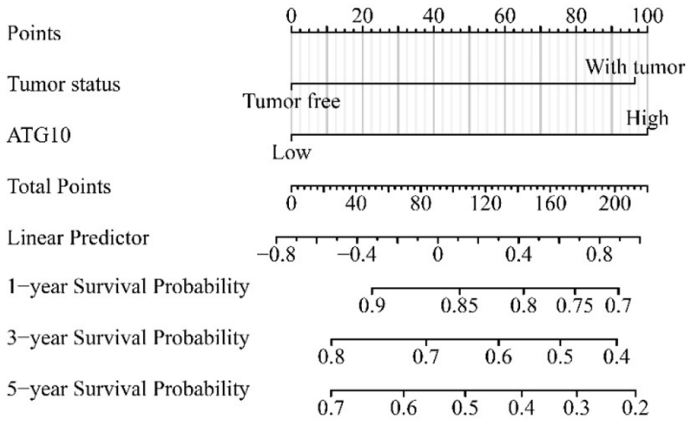


Figure 5. ATG10-related nomogram on overall survival.

with shorter OS, which was consistent with the experimental results showing that the overexpression of ARHGAP9 inhibited the proliferation, migration and invasion of HCC cells. Besides, the expression of transcription factor forkhead box J2 (FOXJ2) was induced by ARHGAP9 overexpression in HepG2 cells, which in turn inhibited the migration and invasion of HepG2 cells. Further mechanistic studies demonstrated that ARHGAP9/FOXJ2 could inhibit HCC growth and migration by inducing cadherin-1 (Cdh1) transcription [18]. As for ATG10, ample studies have suggested that ATG10 plays an important role in the tumorigenesis of cancers such NSCLC, CRC, and gastric cancer [9-14]. For example, the antitumor effect of Podofilox in inhibiting gastric cancer cell proliferation and colony formation was considered through inhibiting the expression of ATG10. Similar findings were also obtained by silencing ATG10 with siRNA, in which siATG10 inhibited the malignant phenotype of gastric cancer cells [13]. Another study found that ATG10 might be one of the hub genes downstream of AP003469.4 mediating AP003469.4-regulated HCC cell growth and migration [2]. Nonetheless, the roles of ATG10 in HCC remain to be fully understood. In this study, we performed bioinformatics analysis and experimental validation to demonstrate that ATG10 was significantly upregulated in HCC tissues, and that ATG10 overexpression correlated with the OS, DSS, and PFI of HCC patients. Furthermore, Cox regression analysis revealed that the elevated ATG10 expression level was an independent risk factor for the OS and DSS of HCC patients, suggesting that the overexpression of ATG0 was a risk factor for the poor prognosis of HCC patients.

Previous studies have shown that ATG10 is involved in the proliferation and migration of cancer cells [12, 14]. For example, Wang et al. reported that Linc00261 was down-regulated in high-grade serous ovarian cancer (HGSOC). Mechanistically, Linc00261 targets the carcinogenic miR-552 and then increases the expression of ATG10 to inhibit the proliferation and migration of HGSOC cells [14]. In addition, Some studies have also reported that changes in cyclin B1, CDK1 and CDK2 expression are related to cancer progression [19-21]. For example, the overexpression of MCTS1 Re-Initiation and Release Factor (MCTS1) was associated with short progression-free survival (PFS) and DSS in laryngeal squamous cell carcinoma (LSCC) patients through promoting LSCC cell viability, colony formation and cell cycle progression via signaling pathways related to the increased expression of CDK1, CDK2, cyclin A2 and cyclin B1 [19]. In our study, we also found that silencing ATG10 expression could suppressed the proliferation and migration of HCC cells via attenuating the cyclin B1/CDK1 and CDK2 signaling pathways. Taken together, our results suggested that ATG10 was involved in HCC progression as an oncogene, and the inhibition of ATG10 expression could reduce HCC progression.

In recent years, several studies have shown that risk models and nomograms could predict the survival of cancer patients [22-24]. Fang et al. constructed a risk model based on the expression autophagy genes to predict that the OS of HCC patients was worse in the high-risk group than in the low-risk group as the low-risk group was involved in autophagy and immune processes. Similarly, a nomogram was constructed based on the expression of autophagy genes to predict the OS in HCC patients [23]. In a risk model constructed based on nine immune genes, the OS was significantly decreased in high-risk patients [24]. In this study, we also constructed nomograms and risk models associated with poor OS, PFI and DSS of HCC patients. Specifically, we found that the expression of ATG10, ATG12, LARS1, CWC27,

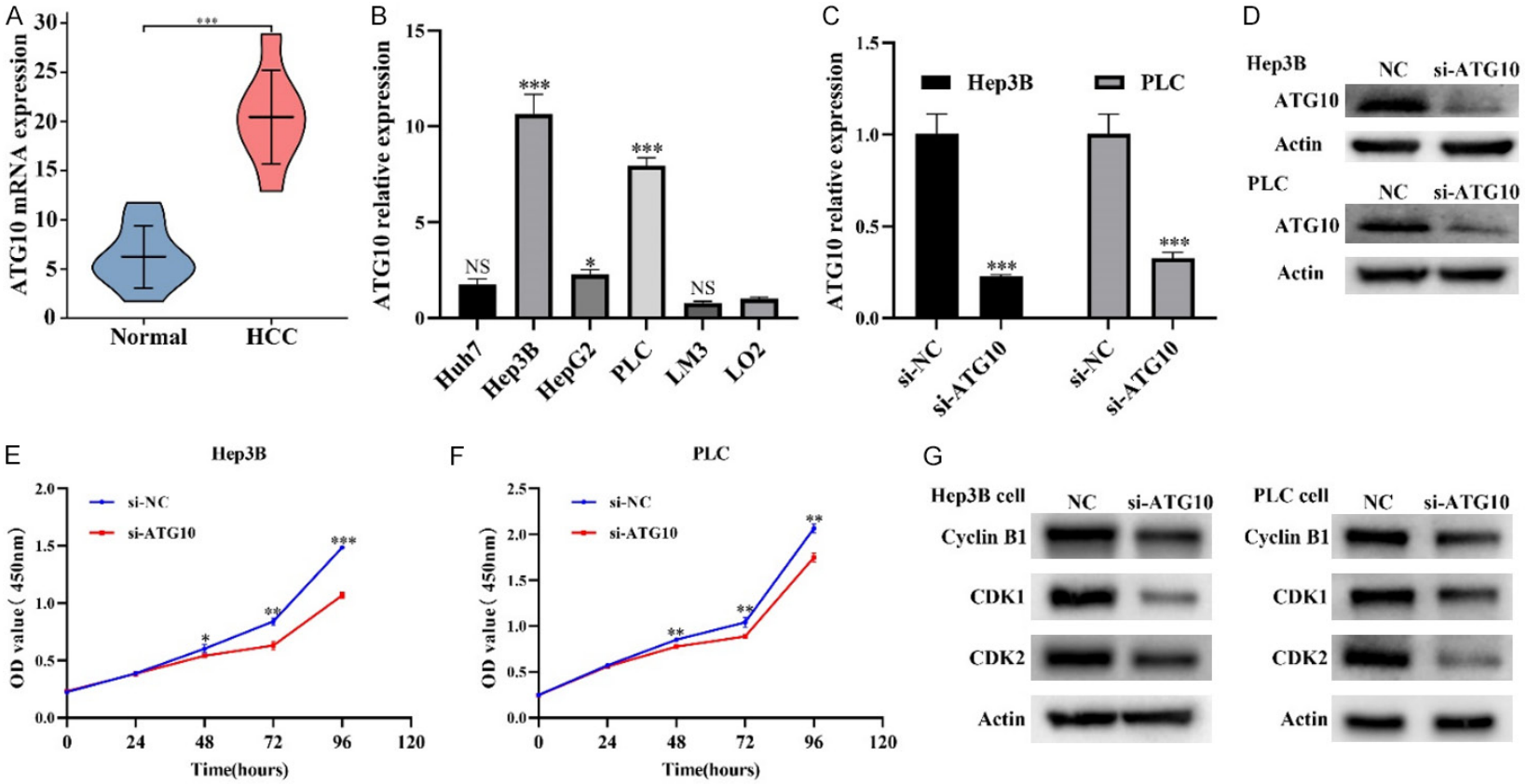


Figure 6. Silencing of ATG10 inhibited cell proliferation through cyclin B1/CDK1 and CDK2. A, B. ATG10 expression in HCC tissues and cells; C, D. Generation of ATG10-knockdown cells by siRNA; E, F. Cell proliferation as determined by CCK-8 assay; G. The expression levels of cyclin B1, CDK1 and CDK2 in ATG10-knockdown cells.

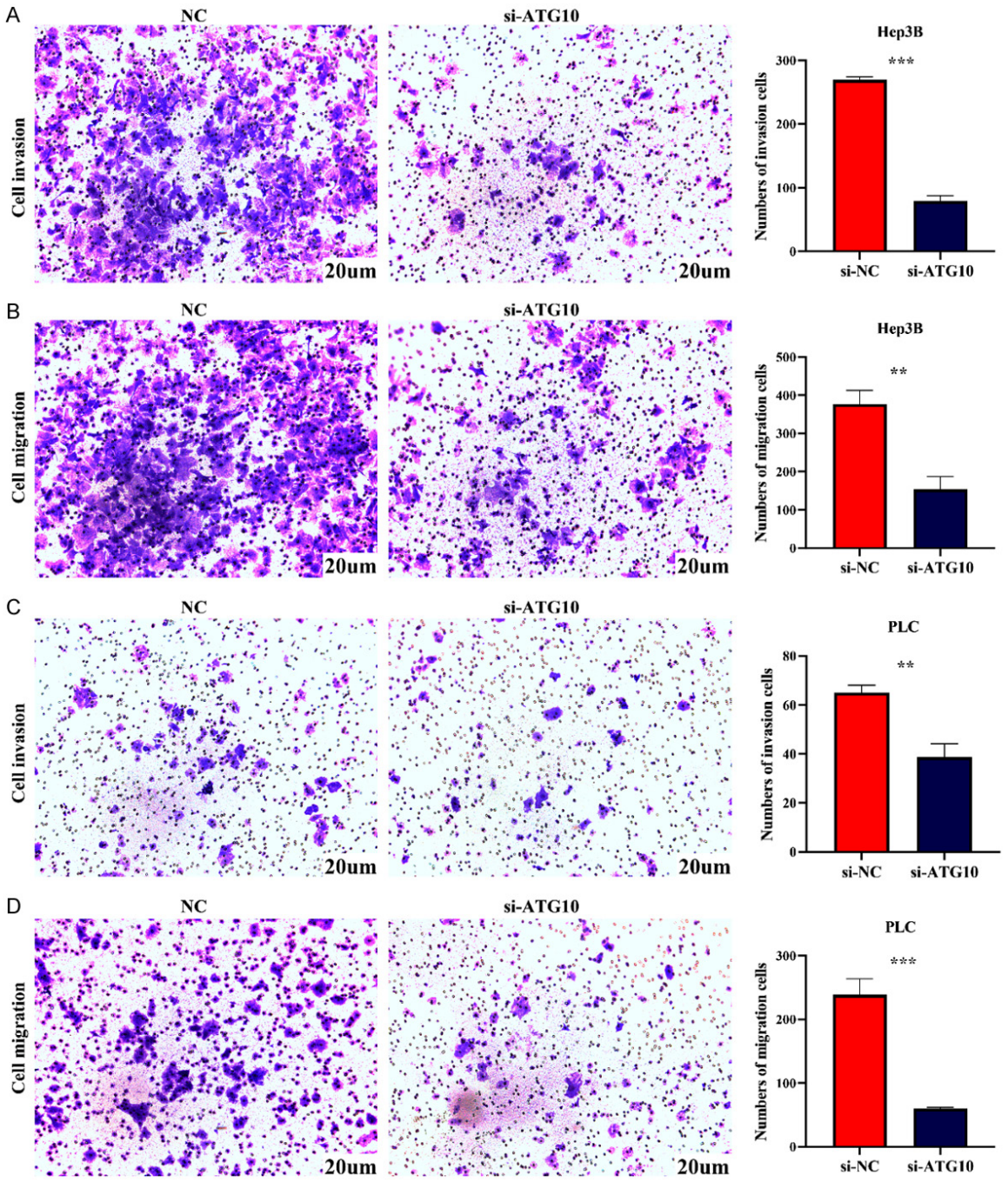


Figure 7. Inhibition of ATG10 expression attenuated the migration and invasion of HCC cells. A, B. Migration and invasion of Hep3B cells; C, D. Migration and invasion of PLC cells.

and SLC30A5 was upregulated in HCC tissues and was significantly associated with a dismal prognosis. Thus, the nomograms constructed based on the expression of ATG10, ATG12, LARS1, CWC27, and SLC30A5 could predict the OS, DSS, and PFI of HCC patients. We also constructed several risk models for prognosis prediction. One risk model was constructed based on the expression of ATG10, LARS1, and CWC27, and the LASSO algorithm demonstrated that HCC patients in the high-risk group had the shorter OS. Another risk model based on the expression of ATG10, ATG12 and CWC27 showed that HCC patients in the high-risk group

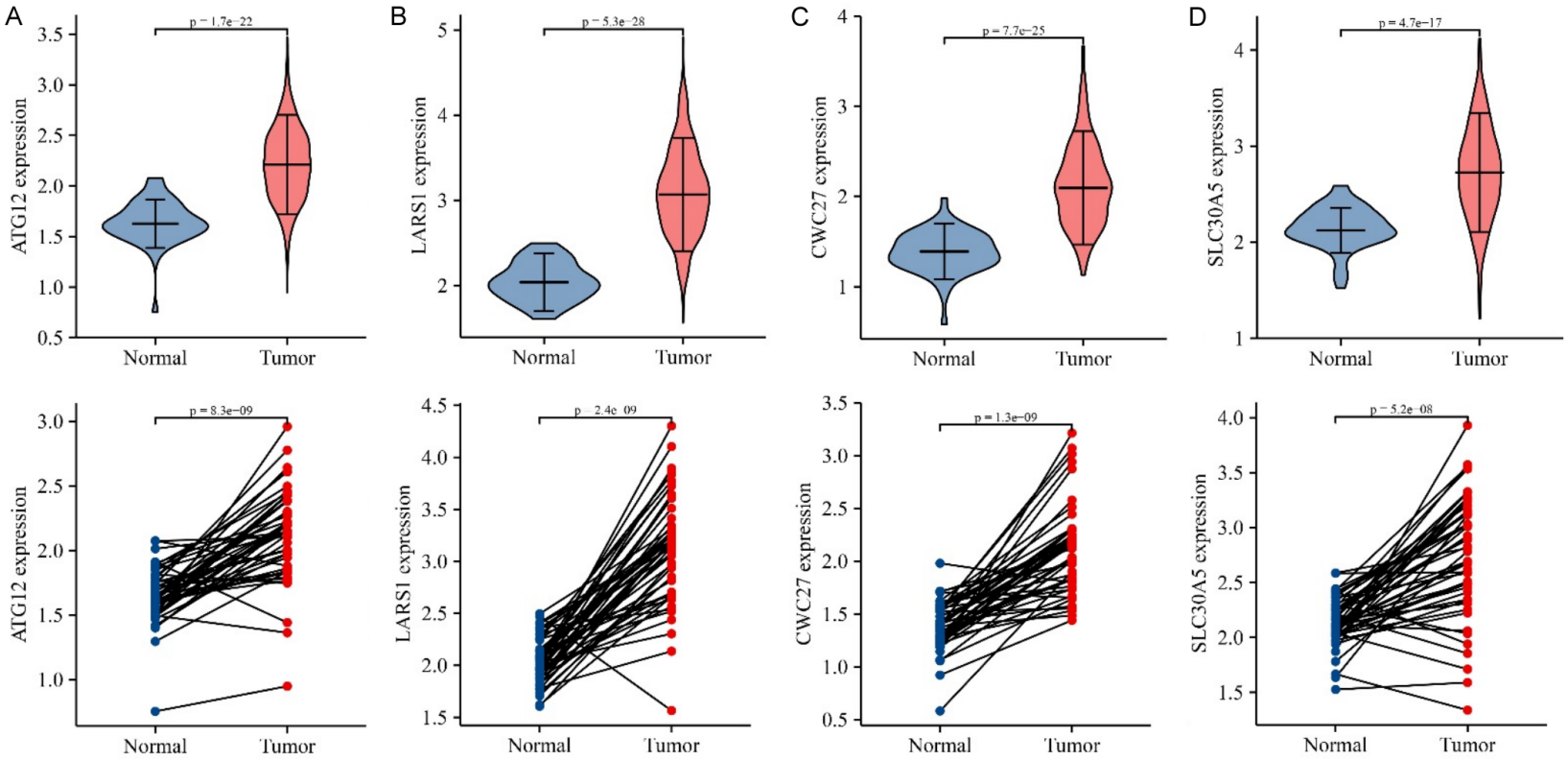


Figure 8. The expression levels of ATG10-overlapping genes in HCC. A. ATG12; B. LARS1; C. CWC27; D. SLC30A5. Note: HCC, hepatocellular carcinoma.

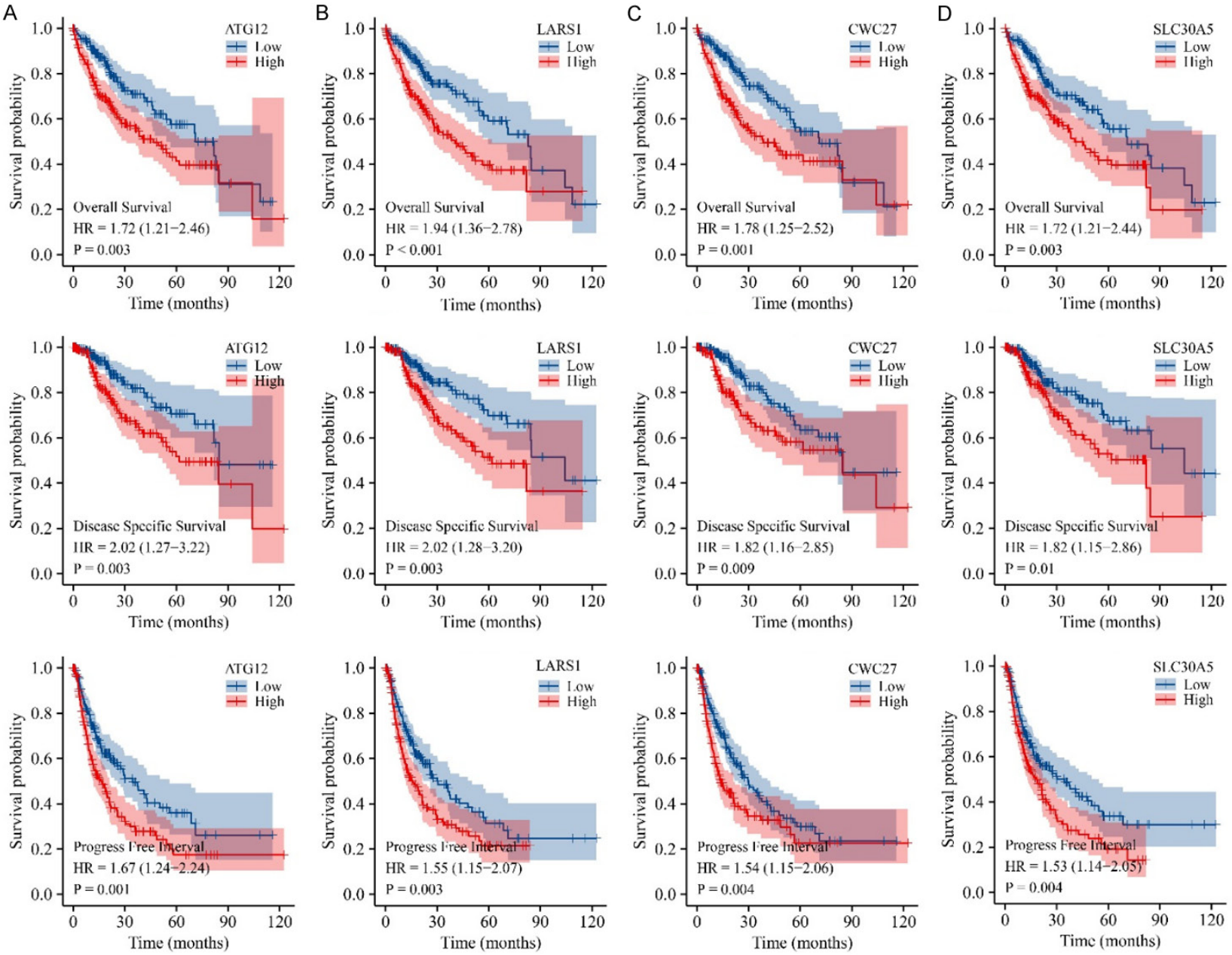

A
ATG12
Low
High
Survival probability
Overall Survival
HR = 1.72 (1.21−2.46)
P = 0.003
Time (months)
B
LARS1
Low
High
Survival probability
Overall Survival
HR = 1.94 (1.36−2.78)
P < 0.001
Time (months)
C
CWC27
Low
High
Survival probability
Overall Survival
HR = 1.78 (1.25−2.52)
P = 0.001
Time (months)
D
SLC30A5
Low
High
Survival probability
Overall Survival
HR = 1.72 (1.21−2.44)
P = 0.003
Time (months)
ATG12
Low
High
Survival probability
Disease Specific Survival
HR = 2.02 (1.27−3.22)
P = 0.003
Time (months)
LARS1
Low
High
Survival probability
Disease Specific Survival
HR = 2.02 (1.28−3.20)
P = 0.003
Time (months)
CWC27
Low
High
Survival probability
Disease Specific Survival
HR = 1.82 (1.16−2.85)
P = 0.009
Time (months)
SLC30A5
Low
High
Survival probability
Disease Specific Survival
HR = 1.82 (1.15−2.86)
P = 0.01
Time (months)
ATG12
Low
High
Survival probability
Progress Free Interval
HR = 1.67 (1.24−2.24)
P = 0.001
Time (months)
LARS1
Low
High
Survival probability
Progress Free Interval
HR = 1.55 (1.15−2.07)
P = 0.003
Time (months)
CWC27
Low
High
Survival probability
Progress Free Interval
HR = 1.54 (1.15−2.06)
P = 0.004
Time (months)
SLC30A5
Low
High
Survival probability
Progress Free Interval
HR = 1.53 (1.14−2.05)
P = 0.004
Time (months)

Figure 9. Prognostic values of ATG10 co-expressed genes ATG12, LARS1, CWC27, and SLC30A5 in HCC using K-M survival analysis. A. ATG12; B. LARS1; C. CWC27; D. SLC30A5. Note: HCC, hepatocellular carcinoma; K-M, Kaplan-Meier.

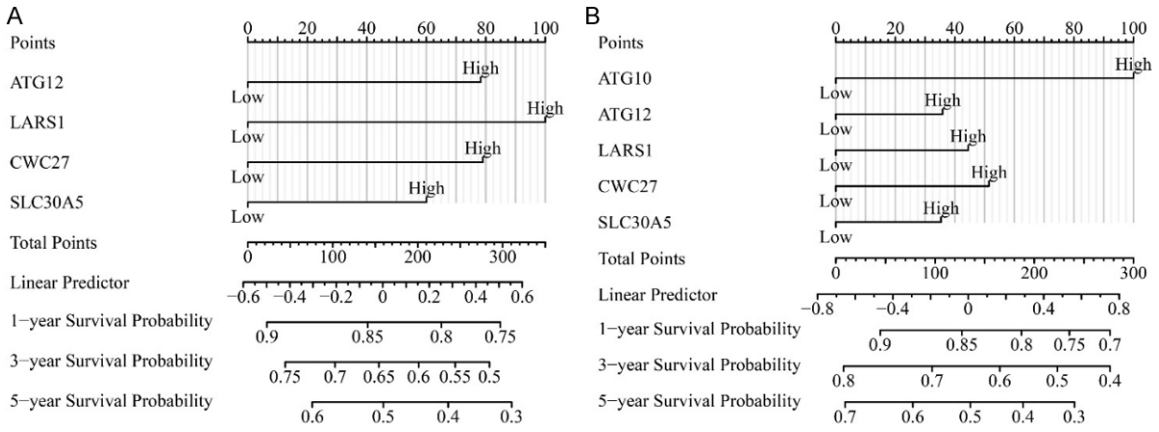


Figure 10. The nomograms of ATG10, ATG12, LARS1, CWC27, and SLC30A5 on OS in HCC. Note: HCC, hepatocellular carcinoma; OS, overall survival.

had a shorter DSS, while the the risk model based on the expression of ATG10, ATG12, CWC27 and SLC30A5 indicated that HCC patients in the high-risk group had a shorter PFI. Collectively, these results suggested that ATG10 could predict the prognosis of patients with HCC.

Although our bioinformatics analysis and the experimental studies indicated the oncogenic roles of ATG10 in HCC, there are several limitations in this study. First, the number of patient samples was small; future studies with larger sample size will further verify our conclusions. Second, the signaling transduction pathways mediating the function of ATG10 in HCC need to be fully elucidated. In conclusion, we found that ATG10 overexpression was significantly correlated with the dismal prognosis of HCC patients. Suppression of ATG10 expression could delay HCC progression. Mechanistically, the elevated expression of ATG10 was involved in autophagy, ubiquitination, and basal transcription factors. Furthermore, the risk models and nomograms constructed based on the expression of ATG10, ATG12, LARS1, CWC27 and SLC30A5 could predict the prognosis of HCC patients, suggesting that ATG10 might serve as a potential biomarker for the diagnosis and prognosis of patients with HCC.

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Disclosure of conflict of interest

None.

Abbreviations

ATG10, Autophagy-related 10; EMT, epithelial-mesenchymal transition; CRC, colorectal cancer; ROC, receiver operating characteristic; HCC, hepatocellular carcinoma; DSS, disease-specific survival; PFI, progression-free interval; OS, overall survival; TCGA, The Cancer Genome Atlas.

Address correspondence to: Rong-Shu Shi and Di Ke, Department of Radiology, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China. E-mail: shirongshujieru@163.com (RSS); kedi94813@163.com (DK); Qiang Guo, Department of Cardiothoracic Surgery, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China. E-mail: guoqianglidan@163.com

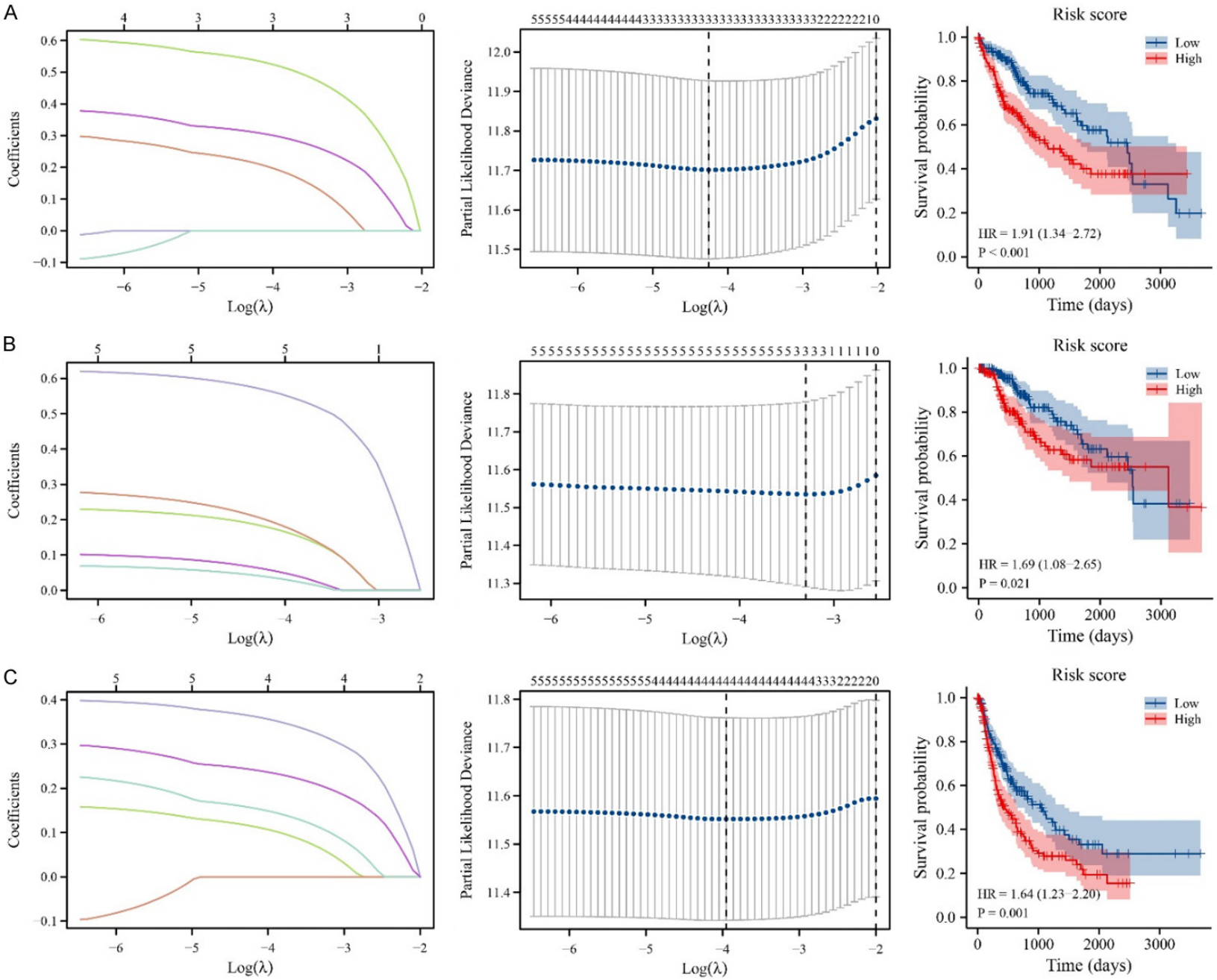
A
Coefficients
Log(λ)
Partial Likelihood Deviance
Log(λ)
Risk score
Low
High
Survival probability
HR = 1.91 (1.34–2.72)
P < 0.001
Time (days)
B
Coefficients
Log(λ)
Partial Likelihood Deviance
Log(λ)
Risk score
Low
High
Survival probability
HR = 1.69 (1.08–2.65)
P = 0.021
Time (days)
C
Coefficients
Log(λ)
Partial Likelihood Deviance
Log(λ)
Risk score
Low
High
Survival probability
HR = 1.64 (1.23–2.20)
P = 0.001
Time (days)

Figure 11. The risk models based on the expression levels of ATG10, and ATG10 co-expressed genes were related to the prognosis in HCC. A. OS; B. DSS; C. PFI. Note: HCC, hepatocellular carcinoma; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

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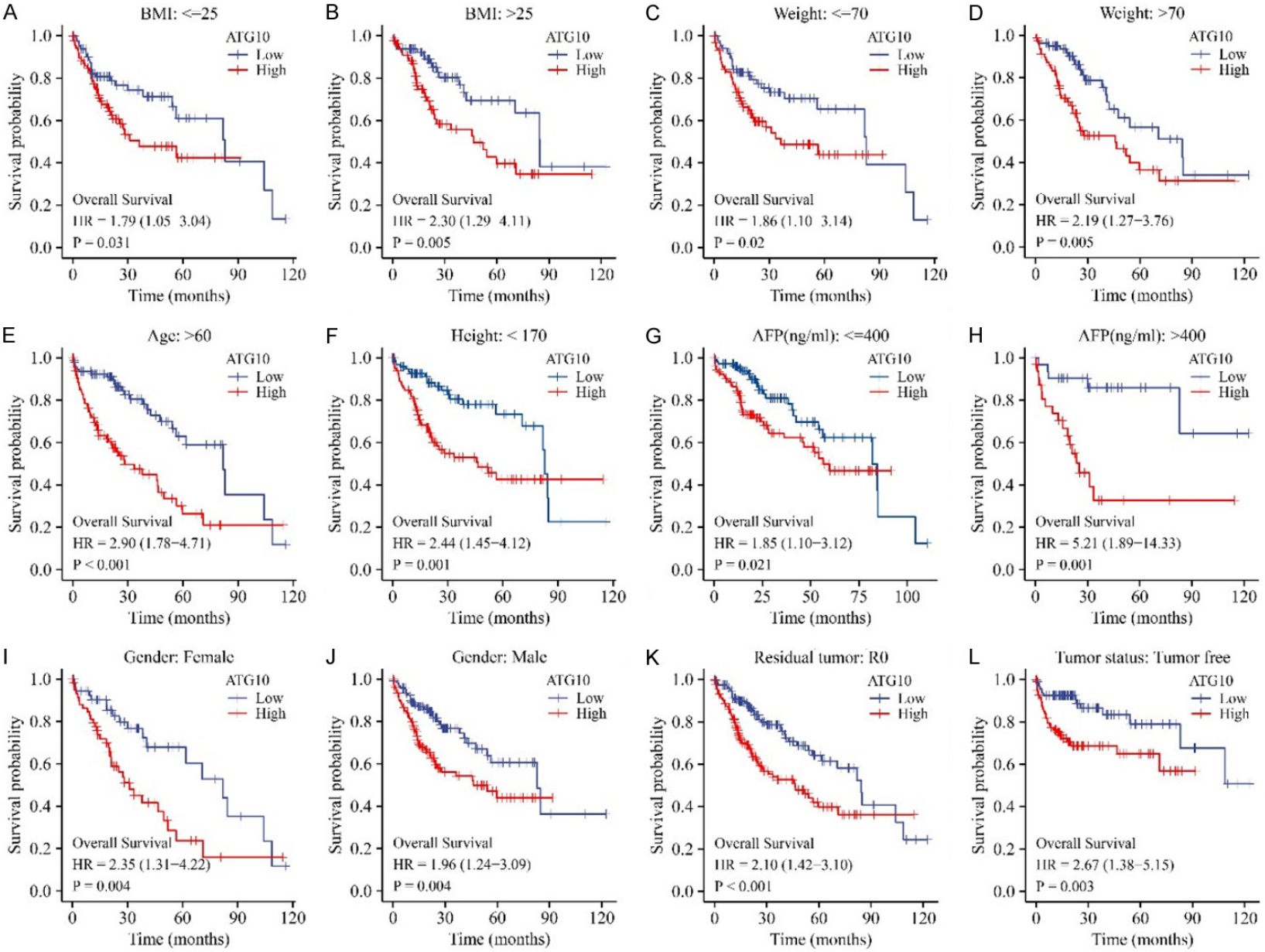
A
BMI: <=25
ATG10
Low
High
Survival probability
Overall Survival
HR = 1.79 (1.05 3.04)
P = 0.031
Time (months)
B
BMI: >25
Overall Survival
HR = 2.30 (1.29 4.11)
P = 0.005
C
Weight: <=70
Overall Survival
HR = 1.86 (1.10 3.14)
P = 0.02
D
Weight: >70
Overall Survival
HR = 2.19 (1.27−3.76)
P = 0.005
E
Age: >60
Overall Survival
HR = 2.90 (1.78−4.71)
P < 0.001
F
Height: < 170
Overall Survival
HR = 2.44 (1.45−4.12)
P = 0.001
G
AFP(ng/ml): <=400
Overall Survival
HR = 1.85 (1.10−3.12)
P = 0.021
H
AFP(ng/ml): >400
Overall Survival
HR = 5.21 (1.89−14.33)
P = 0.001
I
Gender: Female
Overall Survival
HR = 2.35 (1.31−4.22)
P = 0.004
J
Gender: Male
Overall Survival
HR = 1.96 (1.24−3.09)
P = 0.004
K
Residual tumor: R0
Overall Survival
HR = 2.10 (1.42−3.10)
P < 0.001
L
Tumor status: Tumor free
Overall Survival
HR = 2.67 (1.38−5.15)
P = 0.003

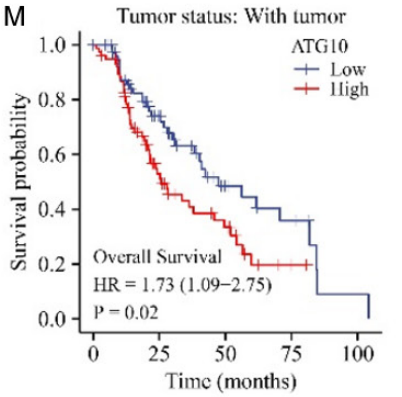

Figure S1. The relationship between ATG10 overexpression and the OS determined by subgroup analysis. A. BMI ≤25; B. BMI >25; C. Weight ≤70; D. Weight >70; E. Age; F. Height; G. AFP ≤400; H. AFP >400; I. Female; J. Male; K. R0; L. Tumor free; M. Tumor status. Note: HCC, hepatocellular carcinoma; OS, overall survival; AFP, alpha-fetoprotein.

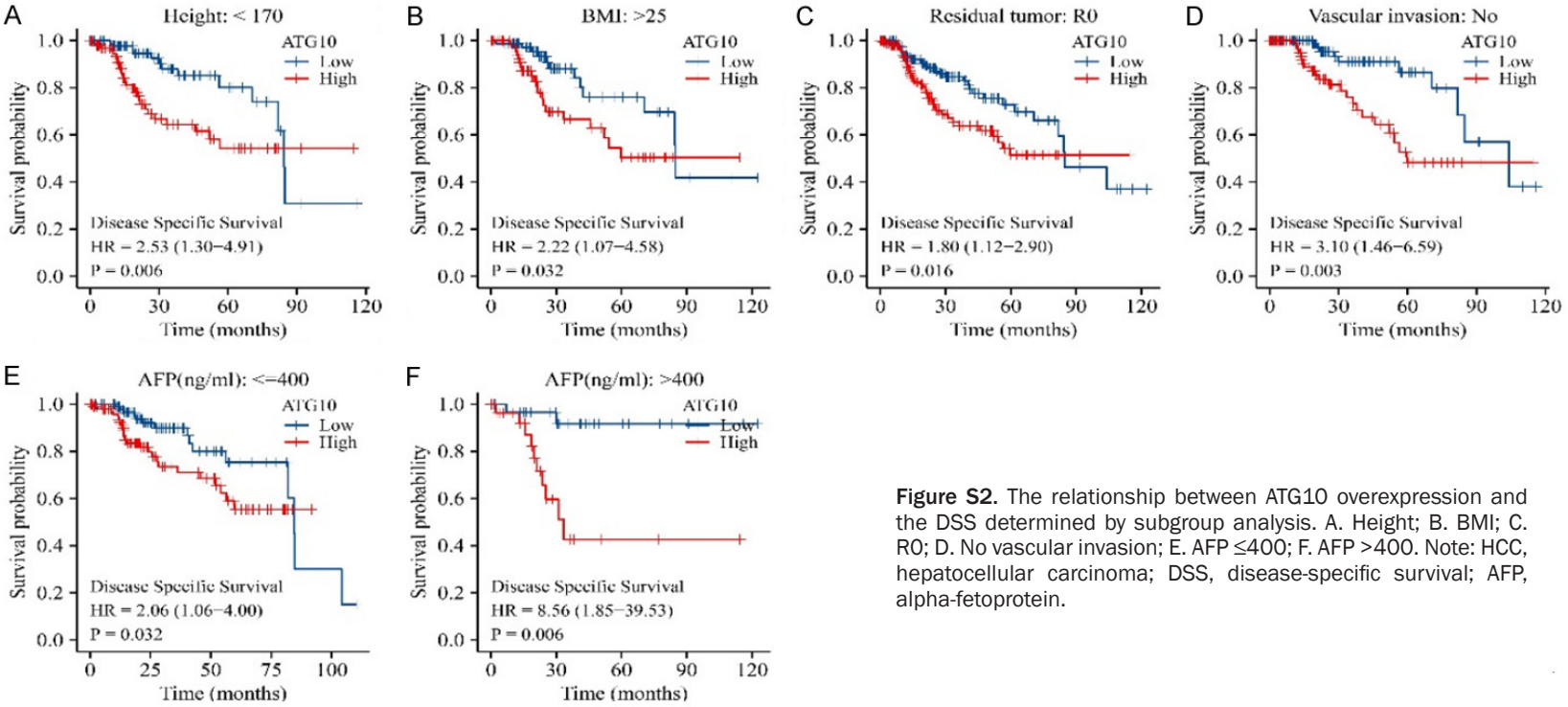

Figure S2. The relationship between ATG10 overexpression and the DSS determined by subgroup analysis. A. Height; B. BMI; C. R0; D. No vascular invasion; E. AFP ≤400; F. AFP >400. Note: HCC, hepatocellular carcinoma; DSS, disease-specific survival; AFP, alpha-fetoprotein.

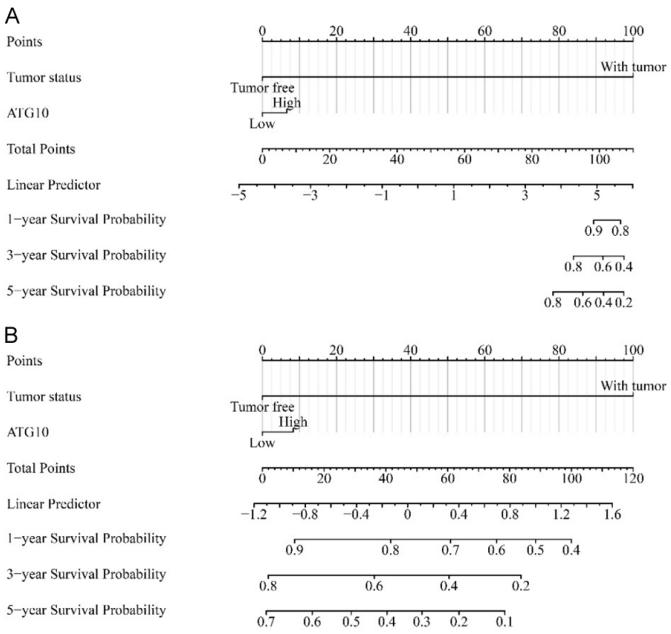

Figure S3. ATG10-related nomograms predicted the DSS and PFI of HCC. A. DSS; B. PFI. Note: HCC, hepatocellular carcinoma; DSS, disease-specific survival; PFI, progression-free interval.

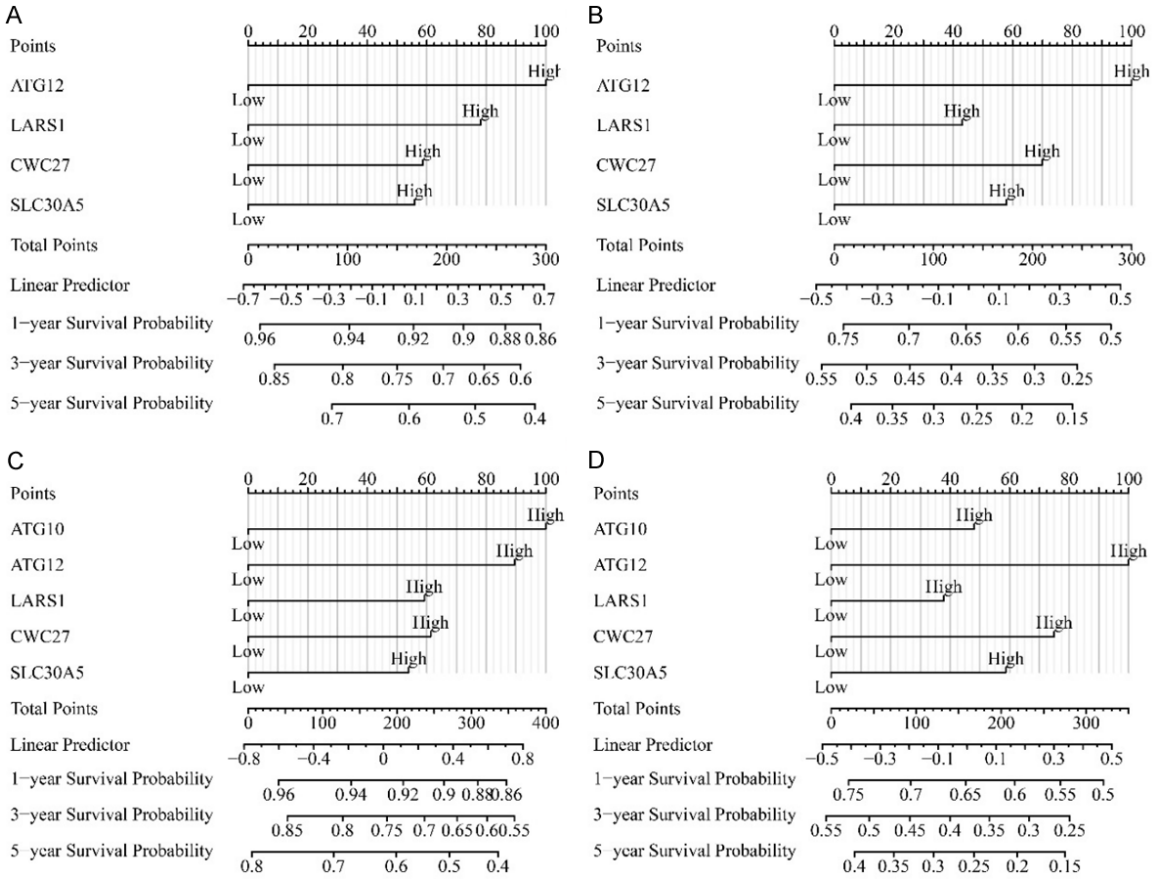

Figure S4. The nomograms based on the expression of ATG10, ATG12, LARS1, CWC27, and SLC30A5 predicted the DSS and PFI in HCC. Note: HCC, hepatocellular carcinoma; DSS, disease-specific survival; PFI, progression-free interval.