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Original Article

Prognostic potential and oncogenic effects of UCH-L1 expression in hilar cholangiocarcinoma

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Abstract: Background: UCH-L1 has been implicated to playing a potential role in cancer development and progression. However, UCH-L1's role in hilar cholangiocarcinoma remains unclear. Methods: The function of UCH-L1 in hilar cholangiocarcinoma was evaluated using human tissues, molecular and cell biology, and animal models, and its prognostic significance was determined according to its impact on patient survival. Results: In the present study, UCH-L1 was overexpressed in 62.1% of patients with primary hilar cholangiocarcinoma. Overexpression of UCH-L1 is associated with large tumor size, advanced tumor stage, lymph node metastasis, advanced TNM stage, and high CA19-9 levels, and is also correlated with poor survival rates. Silencing of UCH-L1 inhibited proliferation, colony formation of hilar cholangiocarcinoma cells in vitro and suppressed tumor growth of hilar cholangiocarcinoma cells in vivo. We also observed that silencing of UCH-L1 decreased the phosphorylation level of Akt and PCNA in the xenograft experiments. Discussion: Taken together, these findings suggest that UCH-L1 functions as an oncogene in the development and progression of hilar cholangiocarcinoma. UCH-L1 can serve as an independent prognostic factor and maybe a potential therapeutic target for patients with hilar cholangiocarcinoma.

Keywords: Hilar cholangiocarcinoma, prognosis, UCH-L1, biomarkers

Introduction

Cholangiocarcinoma, a neoplasm arising from the bile duct epithelium, is the second most common primary liver cancer in most parts of the world [1]. Although there has been some progress in therapy, the prognosis of hilar cholangiocarcinoma is still devastating, and surgical resection or liver transplantation is the only curative therapy [2]. Due to early invasion, widespread metastasis and the lack of an effective therapy, hilar cholangiocarcinoma is an aggressive malignancy and is associated with a high incidence and mortality rate [3, 4]. Therefore, it is essential to study the underlying mechanisms of hilar cholangiocarcinoma progression, explore novel biomarkers in predicting hilar cholangiocarcinoma patient' outcomes and develop effective therapeutic strategies.

Ubiquitin Carboxyl-Terminal Hydrolase-L1 (UCH-L1) is a deubiquitinating enzyme that cleaves the ubiquitin moiety from ubiquitin precursors or protein substrata [5]. Although UCH-L1 was originally characterized as an important enzyme for neurodegenerative diseases, recent studies indicate that it also relates to tumor development [6]. UCH-L1 has been reported as either an oncogene or a tumor suppressor [7]. The correlation between UCH-L1 and tumor has been reported in various tumor tissues, including osteosarcoma [8], prostate cancer [5], non-small cell lung carcinoma [9] and breast cancer [10]. However, the role of UCH-L1 in hilar cholangiocarcinoma has not been reported.

In the study described here, we evaluated the expression of UCH-L1 in resected hilar cholangiocarcinoma specimens, and determined their clinical and prognostic significance in these tumors by correlating their expression with clinicopathologic features and survival. In order to verify the clinical experimental results, hilar cholangiocarcinoma cells were transfected with UCH-L1 shRNA or unspecific scrambled shRNA plasmids. The proliferation, colony for-

mation of hilar cholangiocarcinoma cells, and phosphorylation level of Akt were tested. Finally, subcutaneous tumor xenograft models were performed to evaluate the in vivo function alteration of UCH-L1-knockdown in QBC939 cell line.

Materials and methods

Patient specimens and cell lines

The institutional review boards of both the Nanjing Medical University affiliated Wuxi Second Hospital and the Changhai Hospital approved the use of the patients' samples and clinical information, and each patient or his or her guardian gave informed consent to participate in the study. All the experimental methods in the current study were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki), and approved by the Ethics Committee of the Nanjing Medical University affiliated Wuxi Second Hospital and the Changhai Hospital.

A total of 95 patients with primary hilar cholangiocarcinoma who underwent curative surgery at the Nanjing Medical University affiliated Wuxi Second Hospital and the Changhai Hospital in Shanghai, People's Republic of China, from 2008 to 2014 were enrolled in this study. Histological confirmation of primary hilar cholangiocarcinoma was obtained from the two hospitals. All cases have matched non-neoplastic bile duct tissues which were sampled from the resection margins, where they were the most distant away from the tumor borderline. The patients' medical records were reviewed to obtain patient data, including age at diagnosis, sex, tumor location, tumor size, differentiation, lymph node metastasis, and the American Joint Committee on Cancer stage.

The hilar cholangiocarcinoma cell line QBC939 (purchased from the Cell Center of Chinese Academy of Sciences, Shanghai, China) was cultured in Dullbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Invitrogen Corp., Grand Island, NY). Cell line QBC939 was cultured in a 37°C humidified atmosphere containing 95% air and 5% CO_2.

Tissue microarray construction

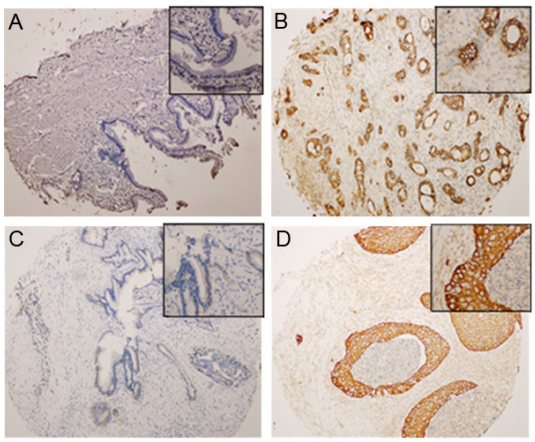
Four paraffin-embedded tissue microarray blocks of normal bile duct tissue and the hilar cholangiocarcinoma tissue obtained from the patients were generated using a manual arrayer (Beecher Instruments, Sun Prairie, WI, USA). Each block had at least one 1.5-mm core of non-neoplastic bile duct tissue and two 1.5-mm cores of hilar cholangiocarcinoma tissue. For patients with lymph node metastases, one or two 1.5-mm cores of metastatic tissue were included. All of the tissue specimens were obtained for the present study with informed patient consent, and the use of human specimens was approved by the Nanjing Medical University affiliated Wuxi Second Hospital and the Changhai Hospital Institutional Review Board.

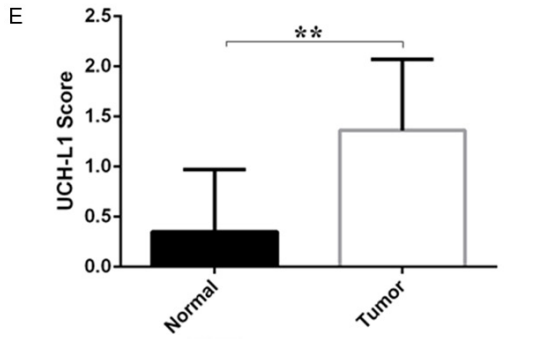
Immunohistochemistry

Four-micron sections of paraffin-embedded specimens were used for immunohistochemical analysis. Slides were deparaffinized in fresh xylene and dehydrated through sequential graded ethanol. Antigen retrieval for UCH-L1 and p-Akt was performed using citrate buffer incubation (10 mmol/L, pH 6) with a microwavable pressure cooker for 20 min. Then, slides were cooled for 20 min, incubated for 5 min with 3% hydrogen peroxide, washed with phosphate-buffered saline-0.1% Triton X-100 (pH 7.6), blocked for 20 min with 20% serum, and incubated with anti-UCH-L1 (1:100, HPA00-5993; SIGMA-ALDRICH, UA) and p-Akt (1:50, 736E11; CST, Beverly, MA) antibodies overnight at 4°C. Slides were washed with phosphate-buffered saline-0.1% Triton X-100 and incubated for 30 min in a 1:200 dilution of biotinylated secondary antibody. An EnVision kit (Dako, Carpinteria, CA, USA) was used to visualize antibody binding, and slides were subsequently counterstained with hematoxylin.

Evaluation of immunostaining

Expression of UCH-L1 in the tissue microarray chips was evaluated under an Olympus CX31 microscope (Olympus Optical, Tokyo, Japan) by two individuals (GZ.Y. and Y.C.). Discrepancies in the scores were resolved by discussion between the two evaluators. The expression of the UCH-L1 protein was evaluated using a semi-quantitative scoring system [11]. Staining for UCH-L1 was graded on a scale of 0-2 (0, no staining or staining intensity less than normal; 1, staining intensity equal to normal; 2, strong staining, more than normal). Only a score of 2 was regarded as overexpression. Interpretations of the positive results were made independent-





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p-Akt	UCH-L1 -	UCH-L1 +	R	P
-	28	6	0.684	0.000
+	8	53		

Figure 1. Representative pictures of the expression of UCH-L1 and p-Akt in cholangiocarcinoma and normal bile duct tissue. (A) Negative expression of UCH-L1 in normal bile duct tissue; (B) Positive staining of UCH-L1 in the cholangiocarcinoma tissue; (C) Negative expression of UCH-L1 in the cholangiocarcinoma tissue; (D) Positive staining of p-Akt in the cholangiocarcinoma tissue; Original magnification of (A-D) 40 ×; Original magnification of small pictures in (A-D) 200 ×. (E) Levels of UCH-L1 expression in cholangiocarcinoma and normal bile duct tissue. **P<0.01; (F) Correlation between UCH-L1 and p-Akt expression in cholangiocarcinoma.

ly by two pathologists, who had been blinded to each other's findings.

Western blot analysis

Whole-cell lysates of hilar cholangiocarcinoma cell line QBC939, cancer specimens and matched non-tumor tissues were prepared for Western blot analysis. Standard Western blotting was performed using a rabbit antibody against human anti-UCH-L1 (1:1000, HPA005993; SIGMA-ALDRICH, Dorset, UK) and p-Akt (1:1000, 736E11; CST, Beverly, MA) an anti-rabbit IgG antibody, which was a horseradish peroxidase linked F (ab')$_2$ fragment (obtained from donkeys) (RPN430, Amersham, USA). Protein sample loading was monitored by probing the same membrane filter with an anti-β-actin antibody (sc-130301, Santa Cruz, USA).

Plasmids and transfections

UCH-L1 shRNA (sc-42304-V) and unspecific scrambled shRNA plasmids were purchased from Santa Cruz Biotechnology. QBC939 cells were digested, and 1 × 10^5 cells were seeded in six well plates. Transfection of shRNA was carried out using Lipofectamine 2000 reagent (Invitrogen, Karlsruhe, Germany) and 5 ng of shRNA plasmid per well according to the manufacturer's instructions.

Cell proliferation assay

Cells were digested and 5000 cells were seeded in 96-well plates at 12 hours after transfection and incubated in medium with 10% FBS. CCK8 assays (Dojindo Kumamoto, Japan) were performed to measure the final results, which were calculated as the ratio of the absorbance at 3 days compared with that at 1 day. The experiment was repeated three times independently.

Colony formation assay

Cells were digested at 12 hours after transfection and seeded in 6-well plates in triplicate at a density of 500 cells/well for 2 weeks at 37°C. The colonies were fixed with methanol/acetone (1:1) and stained with crystal violet. Colonies with cell numbers of more than 50 cells per colony were counted.

Animal models

The animal experiment was authorized according to the Guidance Suggestions for the Care and Use of Laboratory Animals (issued by the Ministry of Science and Technology of the People's Republic of China), and was approved by the Ethics Committee of the Nanjing Medical University affiliated Wuxi Second Hospital and the Changhai Hospital. Subcutaneous tumor xenograft models were performed to evaluate the in vivo function alteration of UCH-L1-

Table 1. Correlation of the UCH-L1 overexpression and clinicopathological variables of cholangiocarcinoma

Variables	N	UCH-L1 Positive (%)	*P*
Age			
≤60 y	52	32 (61.5)	0.535
>60 y	43	27 (62.8)	
Gender			
Male	68	40 (58.8)	0.295
Female	27	19 (70.4)	
Tumor size			
≤6 cm	33	15 (45.5)	0.015
>6 cm	62	44 (71.0)	
Nerve invasion			
Yes	50	32 (64.0)	0.688
No	45	27 (60.0)	
T stage			
T1/2	14	4 (28.6)	0.005
T3/4	81	55 (67.9)	
N stage			
N0	32	14 (43.8)	0.009
N1-3	63	45 (71.4)	
Differentiation			
Well/moderate	72	46 (63.9)	0.526
Poorly/undifferentiated	23	13 (56.5)	
TNM stage			
I/II	37	15 (40.5)	0.001
III/IV	58	44 (75.9)	
CA199			
Low	49	23 (46.9)	0.002
High	46	36 (78.3)	

knockdown in the QBC939 cell line. UCH-L1-KO QBC939 cells and controls (1×10^6 QBC939 cells in 0.1 mL PBS) were injected subcutaneously into the left flank of 4-week-old female BALB/c nude mice (the Animal Center of the Nanjing Medical University) (n=5). Two weeks after implantation, all animals were sacrificed and tumor weight was measured. HE staining for tissue analysis, immunohistochemistry staining for expression of UCH-L1 (1:100, HPA-005993; SIGMA-ALDRICH, UA), p-AKT (1:50, 736E11; CST, Beverly, MA) and proliferating cell nuclear antigen (PCNA) (1:4000, ab18197; Abcam, UK) were performed.

Statistical analysis

Categorical data were analyzed using χ^2 statistics tests. The within-group correlations of the continuous and ordinal variables were assessed using Pearson's R correlation coefficient or Spearman's correlation coefficient when appropriate. Survival curves were calculated according to the Kaplan-Meier method. The survival data shown in this study pertain to disease-free survival (DFS) and overall survival (OS). Differences between survival curves were examined with the log-rank test. Multivariate analysis of prognostic factors related to overall survival was carried out using Cox's proportional hazards model and a stepwise procedure. The accepted level of significance was $P<0.05$. Statistical analyses and graphics were performed with the SPSS 13.0 statistical package (SPSS, Inc., Chicago, IL).

Results

Correlation between clinicopathological features and UCH-L1 proteins expression

UCH-L1 positive staining was preferentially cytoplasm and nuclear localized (**Figure 1**). The epithelium in hilar cholangiocarcinoma specimens showed moderate or strong UCH-L1 staining. There were 59 cases (62.1%) that showed positive staining of UCH-L1 and the other 36 cases (37.9%) showed negative staining. Importantly, we detected high expression of UCH-L1 in tumor tissues where size >6 cm, but drastically reduced UCH-L1 expression in tumor tissues where size ≤6 cm. We also observed that the expression of UCH-L1 varied with the different lymph node metastasis stage (no lymph node metastasis 43.8% vs lymph node metastasis 71.4%), tumor stage (T1/2 stage 28.65% vs T3/4 stage 67.9%) and TNM stage (TNM stage I/II 40.5% vs TNM stage I/II 75.9%). Meanwhile, UCH-L1 expression was closely connected to the CA19-9 levels (P= 0.002). In the case of a high level of CA19-9, the positive rate of UCH-L1 was 78.3%, but at a low level of CA19-9, the positive rate of UCH-L1 was 46.9%. These results are presented in **Table 1**.

Relationship of UCH-L1 and p-Akt expression

There were 61 cases (64.2%) that showed positive staining of p-Akt and the other 34 cases (35.8%) showed negative staining. Co-expression analysis was performed in H hilar cholangiocarcinoma tumor samples using immunohistochemistry. The IHC data confirmed that UCH-L1 expression was positively associated with

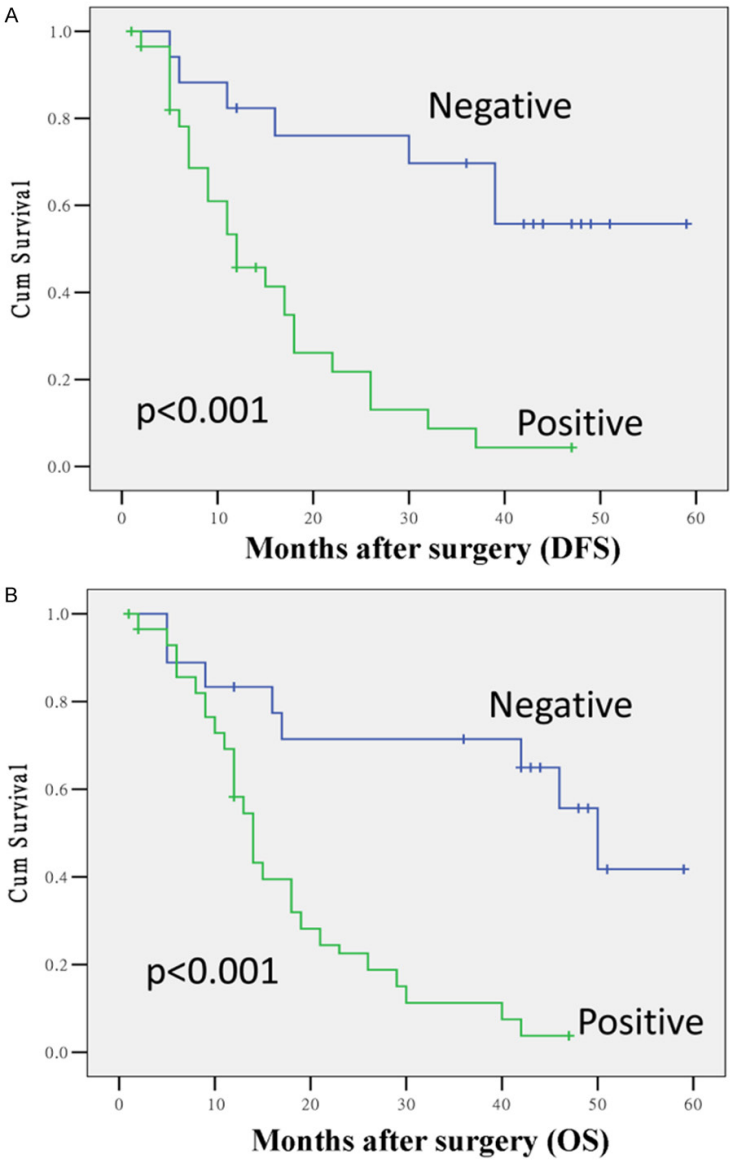

Figure 2. Relationship of UCH-L1 expression with survival of hilar cholangiocarcinoma patients. A. Kaplan-Meier curves of disease free survival durations in patients with cholangiocarcinoma for UCH-L1 expression; B. Kaplan-Meier curves of overall survival durations in patients with cholangiocarcinoma for UCH-L1 expression.

p-Akt expression (r=0.684, P<0.001) in the hilar cholangiocarcinoma tissues (**Figure 1F**).

Relationship of UCH-L1 expression with poor outcome in hilar cholangiocarcinoma patients

A cohort consisted of 68 male (71.6%) and 27 female (28.4%) patients with a median age of 57 years (range 21-87 years). The median cumulative survival duration in patients with resected hilar cholangiocarcinoma was 18 months. Factors that influenced mean survival in the DFS analysis were UCH-L1 (P=0.000), nerve invasion (P=0.031), lymph node metastasis stage (P=0.002), differentiation (P=0.014), margins positive/negative (P=0.005), CA19-9 levels (P=0.000), tumor stage (P=0.000) and TNM stage (P=0.007). Factors that influenced mean survival in the OS analysis were UCH-L1 (P=0.000), lymph node metastasis stage (P=0.001), margins positive/negative (P=0.004), CA19-9 levels (P=0.000), TNM stage (P=0.037), differentiation (P=0.012) and tumor stage (P=0.000). The data for UCH-L1 expression in the DFS and OS analyses are presented in the **Figure 2A** and **2B**.

Multivariate analysis using the Cox proportional hazards model for the DFS of hilar cholangiocarcinoma showed that UCH-L1 expression (P=0.000), nerve invasion (P=0.010), differentiation (P=0.039), and tumor stage (P=0.007) were independent prognostic factors (**Table 2**). Multivariate analysis using the Cox proportional hazards model for the OS of hilar cholangiocarcinoma showed that UCH-L1 expression (P=0.000), differentiation (P=0.039), TNM stage (P=0.043) and tumor stage (P=0.003) were independent prognostic factors (**Table 3**).

Knockdown of UCH-L1 by shRNA inhibits proliferation and colony formation of QBC939 cells in vitro and tumor growth in vivo

We later explored the influence of the expression of UCH-L1 on QBC939 cells by knocking down UCH-L1 (UCH-L1-KO) using specific UCH-L1-shRNA. The effects of UCH-L1-KO were confirmed using Western blot analysis. A significant depletion of UCH-L1 in the transfected cells was observed at 3 days (**Figure 3A**). Then, the influence of UCH-L1-KO on cellular proliferation and colony formation was evaluated. We found down-regulation of UCH-L1 was involved in a

Table 2. Cox regression model for DFS for cholangiocarcinoma

	B	SE	Wald	Sig.	Exp (B)	95.0% CI
Nerve invasion (no *vs* yes)	-.735	.286	6.594	.010	.480	0.274-0.840
T stage (T1/2 *vs* T3/4)	-2.155	.800	7.250	.007	.116	0.024-0.556
N stage (N0 *vs* N1-3)	-.594	.371	2.558	.110	.552	0.267-1.143
Differentiation (well/moderate *vs* poorly/undifferentiated)	-.620	.301	4.250	.039	.538	0.298-0.970
Positive margins (no vs yes)	.234	.331	.502	.479	1.264	0.661-2.416
TNM (I/II *vs* III/IV)	.515	.343	2.246	.134	1.673	0.853-3.281
UCH-L1 (pos. *vs* neg.)	-1.747	.411	18.066	.000	.174	0.078-0.390
CA199 level (low vs high)	-.454	.302	2.262	.133	.635	0.351-1.148

Table 3. Cox regression model for OS for cholangiocarcinoma

	B	SE	Wald	Sig.	Exp (B)	95.0% CI
T stage (T1/2 *vs* T3/4)	-1.772	.602	8.652	.003	.170	0.052-0.554
N stage (N0 *vs* N1-3)	-.562	.349	2.590	.108	.570	0.287-1.130
Differentiation (well/moderate *vs* poorly/undifferentiated)	-.604	.292	4.280	.039	.547	0.309-0.969
Positive margins (no vs yes)	.101	.308	.108	.742	1.107	0.605-2.025
TNM (I/II *vs* III/IV)	.647	.320	4.096	.043	1.910	1.021-3.573
UCH-L1 (pos. *vs* neg.)	-1.419	.368	14.900	.000	.242	0.118-0.497
CA199 level (low vs high)	-.564	.299	3.552	.059	.569	0.316-1.023

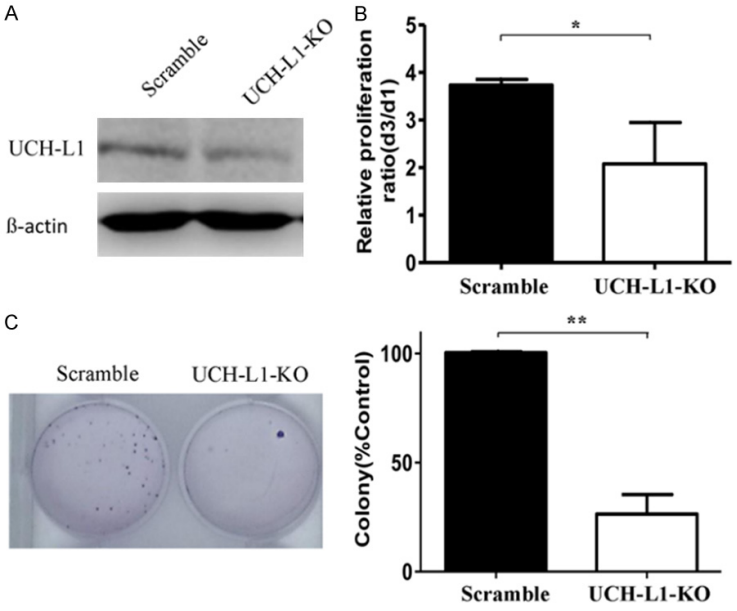

Figure 3. Silencing of UCH-L1 by specific UCH-L1-shRNA inhibits cholangiocarcinoma cells QBC939 proliferation and colony formation. A. The QBC939 cells were transfected with UCH-L1-shRNA for 3 days and Western blotting was used to detect the expression status of UCH-L1; B. Compared with control, the silencing of UCH-L1 inhibits proliferation of QBC939 cells. *P<0.05; C. Silencing of UCH-L1 inhibits the colony forming ability of QBC939 cells in vitro. **P<0.01. The numbers of the cell colonies (>50 cells) were obtained and calculated as: colonies/500 × 100.

dramatic decrease in both cell proliferation (**Figure 3B**) and cell colony formation (**Figure 3C**) in the QBC939 cell line. The in vivo results suggested that silencing of UCH-L1 inhibited tumor growth (**Figure 4**) and decreased the expression of PCNA of hilar cholangiocarcinoma cell lines in vivo (**Figure 5**).

UCH-L1-knockdown downregulated p-Akt expression

We performed western blot analysis to explore whether the expression of Akt and p-Akt was altered in UCH-L1-KO QBC939 cells. The results demonstrated that compared to controls, p-Akt was downregulated and Akt was upregulated in UCH-L1-KO cells (**Figure 6**). The in vivo results were similar to the in vitro results. The expression of p-Akt was lower in the UCH-L1-KO group than in the scramble group (**Figure 5**).

Discussion

The present study indicated that UCH-L1 is a driver of tumorigenesis and can be a predictive

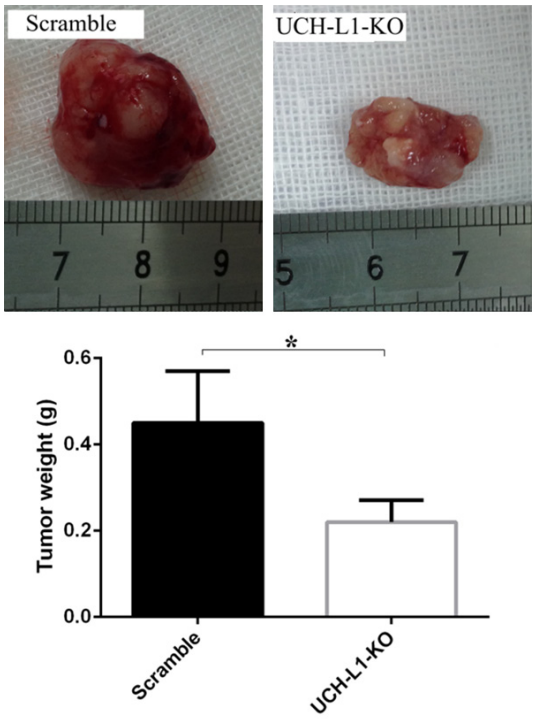

Figure 4. Silencing of UCH-L1 inhibited cholangiocarcinoma growth in vivo, *P<0.05.

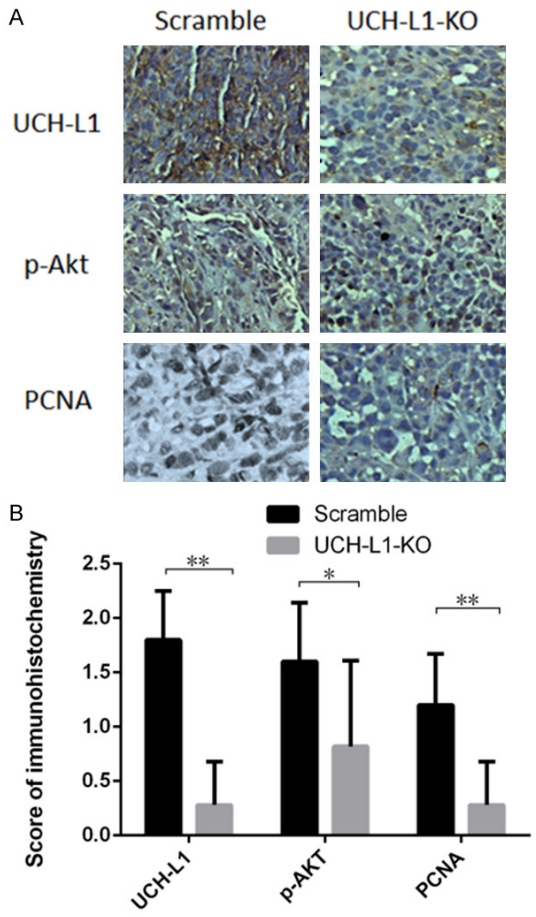

Figure 5. Expression of UCH-L1, p-Akt and PCNA in the UCH-L1-KO and scramble tissues by the method of immunohistochemistry. A. Representative pictures of the expression of UCH-L1, p-Akt and PCNA in the UCH-L1-KO and scramble tissue (original magnification 40 ×); B. Levels of UCH-L1, p-Akt and PCNA expression in the UCH-L1-KO and scramble tissues; **P<0.01 and *P<0.05.

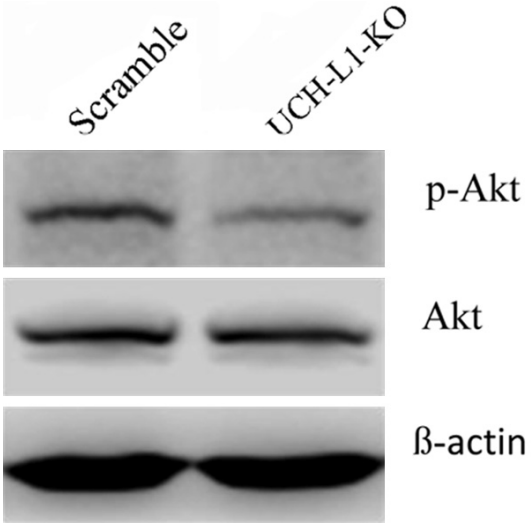

Figure 6. Western blot analysis of Akt and p-Akt expression at 3 days after transfection of UCH-L1-shRNA in QBC939 cells.

factor for the prognosis of hilar cholangiocarcinoma. UCH-L1 was highly expressed in hilar cholangiocarcinoma, and it' s overexpression was closely related to the malignant behavior of hilar cholangiocarcinoma (such as tumor size, tumor stage, lymph node metastasis and TNM stage). Moreover, overexpression of UCH-L1 was significantly associated with poor outcome in patients with hilar cholangiocarcinoma. Down-regulation of UCH-L1 inhibited cell proliferation, colony formation of QBC939 cells and tumor growth of hilar cholangiocarcinoma. UCH-L1 expression was positively related to p-Akt expression, and silencing UCH-L1 reduced the level of p-Akt and PCNA expression.

UCH-L1 is involved in nearly all areas of cell biology and has long been implicated in cancer biology [12, 13]. HJ Kim et al. reported that UCH-L1 is highly expressed in non-small lung cancer cells, and the expression of UCH-L1 in tumor cells enhances their invasive potential in vitro and in vivo [14]. This finding is in agreement with the results of the present study. We observed that UCH-L1 was not expressed or lower expressed in normal bile duct tissues but

overexpressed in hilar cholangiocarcinoma specimens, a difference that was significant. Overexpression of UCH-L1 was associated with the malignant behavior of hilar cholangiocarcinoma, such as large size tumor, advanced tumor stage, lymph node metastasis, and advanced TNM stage. These results suggest that overexpression of UCH-L1 maybe a late event in the development of hilar cholangiocarcinoma.

The risk factors for hilar cholangiocarcinoma have been explored in numerous studies, including levels of total bilirubin, tumor size, lymph node metastases, histologic, neural invasion, depth of cancer invasion and some molecular markers [15, 16]. In the present study, we confirmed that UCH-L1, neural invasion, differentiation and tumor stage were independent predictors for the DFS of hilar cholangiocarcinoma. UCH-L1, differentiation, TNM stage and tumor stage were independent predictors for the OS. There is some controversy about the role of UCH-L1 in estimating the prognoses of tumor patients. UCH-L1 status was not significantly associated with patient outcome, despite the fact that it appears to have an oncogenic role in lung carcinogenesis in the cell line study [9]. However, Y Miyoshi et al. reported that UCH-L1 high expression in breast cancer is associated with a poor prognosis, which suggests the possible involvement of UCH-L1 in the pathogenesis and progression of breast cancer [17]. Our data, from the univariate and multivariate survival analysis, suggest that UCH-L1 expression significantly correlated with prognosis. These findings indicated that overexpression of UCH-L1 is an independent poor prognostic factor of hilar cholangiocarcinoma.

UCH-L1 is a multi-functional molecule, and its mechanism of tumorigenesis involves a complex process. The epithelial-to-mesenchymal transition (EMT) is an important process for cancer cell invasion and metastasis, and the mesenchymal-to-epithelial transition (MET) is the reverse of EMT [18]. The expression of UCH-L1 promotes EMT in prostate cancer cells with a benign biological behavior [5]. Knockdown of UCH-L1 induces MET in prostate cancer cells with a malignant biological behavior, then decreases the migration and invasiveness of tumor cells [5]. Some studies have implicated UCH-L1 as a cell-cycle regulator that relates to cell growth, apoptosis, and transcriptional regulation [19-21]. UCH-L1 expression might contribute to the pathogenesis and progression of cancers through enhanced degradation of p27 (an inhibitor of G1 cyclin-dependent kinases) [22]. The increased expression of UCH L1 in malignant cells leads to cell cycle progression, increased proliferation and migration and at the same time abrogation of apoptotic pathways and immune responses and thus promotes tumor progression [23].

UCH-L1 is an upstream regulator of Akt [24, 25]. Akt is commonly expressed in hilar cholangiocarcinoma, and its expression relates to the prognosis of hilar cholangiocarcinoma patient [26]. Recently, research from Hussain et al. showed that UCH-L1 transgenic mice are prone to malignancy, primary lymphomas and lung tumors, and that UCH-L1 hyperactivity deregulates normal Akt signaling [13]. Similarly, in the present study, UCH-L1 expression was positively associated with p-Akt expression, and p-Akt level was down-regulated after transfection with UCH-L1-shRNA in QBC939 cells. These results provided a molecular basis for cross-talk between Akt and UCH-L1. Taken together, the cross-talk between Akt and UCH-L1 might play a critical role in hilar cholangiocarcinoma cell growth and tumor metastasis.

Conclusion

To the best of our knowledge, there are limited reports demonstrating the clinicopathological significance of UCH-L1 expression in hilar cholangiocarcinoma. Our results indicate that UCH-L1 overexpression is associated with important clinicopathological features and poor outcome in patients with hilar cholangiocarcinoma. Down-regulation of UCH-L1 inhibits the proliferation and colony formation of QBC939 cells and decreases the expression level of p-Akt and PCNA. The cross-talk between UCH-L1 and p-Akt provides a novel clue for exploring prognostic biomarkers and potential therapeutic targets for hilar cholangiocarcinoma. UCH-L1 functions as an oncogene in the development and progression of hilar cholangiocarcinoma.

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Disclosure of conflict of interest

None.

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