Original Paper

Long Noncoding RNA AFAP1-AS1 Promoted Tumor Growth and Invasion in Cholangiocarcinoma

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Key Words

Long non-coding RNAs • AFAP1-AS1 • CCA • Proliferation • Invasion

Abstract

Background: Long non-coding RNAs (lncRNAs) have been shown to play important roles in a wide range of pathophysiological processes, including cancer progression. Our previous study has shown that AFAP1-AS1 was upregulated and acted as an oncogene in hepatocellular carcinoma. However, the expression and biological functions of lncRNA AFAP1-AS1 in intrahepatic cholangiocarcinoma (CCA) remains largely unknown. **Methods:** The expression level of AFAP1-AS1 was measured in 56 pairs of human cholangiocarcinoma tumor tissues and corresponding adjacent normal bile duct tissues. The correlation between AFAP1-AS1 and the clinicopathological features were evaluated by chi-square test. The effects of AFAP1-AS1 on CCA cells were determined by CCK-8 assay, clone formation assay, flow cytometry and transwell assay. Finally, to determine the effect of AFAP1-AS1 on tumor growth *in vivo*, AFAP1-AS1 knockdown CCLP-1 cells were subcutaneously into nude mice to evaluate tumor growth. **Results:** In this study, we found that lncRNA AFAP1-AS1 was increased in CCA tissues and patients with high AFAP1-AS1 expression had a shorter overall survival. SiRNA-mediated AFAP1-AS1 knockdown significantly decreased cell proliferation of the CCA cells, with downregulation of C-myc and Cyclin D1 *in vitro*. Furthermore, AFAP1-AS1 silencing inhibited cell migration partly due to decrease the expression of MMP-2 and MMP-9. In addition, CCLP-1 cells with AFAP1-AS1 knockdown were injected into nude mice to investigate the effect of AFAP1-AS1 on the tumorigenesis *in vivo*. **Conclusions:** Taken together, our findings suggested that AFAP1-AS1 might promote the CCA progression and provided a novel potential therapeutic target for CCA.

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Introduction

Cholangiocarcinoma (CCA) was arisen from biliary epithelial cells lining the bile duct and the second most common primary hepatic carcinoma [1, 2]. The incidences and mortality rates have increased due to delays in diagnosis during the past decades, especially people in

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Asia and Latin America [3, 4]. Liver fluke, primary sclerosing cholangitis, hepatolithiasis, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection were the known risk factors for CCA [5-7]. Despite great advancement of surgical resection and conventional chemotherapeutic agents, patients with CCA have low overall survival rates and poor prognosis [2, 3], indicating that new and effective strategies were required to explore the earlier diagnosis of CCA and to develop therapeutic strategies.

Growing evidence indicated that long non-coding RNAs (lncRNAs), a type of endogenous small noncoding RNAs, played an important role in various cellular and developmental processes [8-10]. Aberrant expression of lncRNAs has been shown to be involved in various disease pathogenesis, such as gastric cancer, hepatocellular carcinoma, breast cancer, lung cancer, and CCA [11-14]. Several reports have shown that dysfunction of lncRNAs were associated with CCA. For example, overexpression of lncRNAs H19 and HULC promoted CCA cell migration and invasion by regulating IL-6 and CXCR4, respectively [15]. lncRNA CPS1-IT1 was observed to be upregulated in intrahepatic cholangiocarcinoma and knockdown of CPS1 CPS1-IT1 decreased the proliferation and induced the apoptosis of ICC-9810 cells [16]. lncRNA AFAP1-AS1 (actin filament associated protein 1 antisense RNA1), has been reported to be upregulated in esophageal adenocarcinoma, hepatocellular carcinoma, pancreatic ductal adenocarcinoma, and nasopharyngeal carcinoma [17-20]. However, the expression and detailed function of AFAP1-AS1 in CCA remains largely unclear and needs to be investigated.

In this study, we sought to determine the expression of AFAP1-AS1 in CCA tissues and paired noncancerous tissues and the relationship between the expression of AFAP1-AS1 and clinical characteristics. Further functional studies revealed that knockdown AFAP1-AS1 could result in inhibition of cell proliferation and invasion *in vitro* and tumor growth *in vivo*.

Materials and Methods

Tumor and adjacent tissue samples

The 56 pairs of human tumor tissues and corresponding adjacent normal bile duct tissues were obtained from patients diagnosed with CCA who underwent resection in the First Affiliated Hospital of Zhengzhou University between 2008 and 2012. No previous local or systemic treatment had been conducted on these patients before surgery. Written informed consent was obtained from all study participants and the study was approved by the Institutional Review Boards of the First Affiliated Hospital of Zhengzhou University. All collected specimens were immediately frozen in liquid nitrogen and stored at -80°C until use.

RNA extraction, reverse transcription, and quantitative real-time PCR

The detailed procedure was performed as our previous described [19]. In brief, total RNA was extracted from cells using Trizol Reagent (Invitrogen). Reverse transcription of 500 ng of RNA was done using PrimeScript™ RT Master Mix (TAKARA, Dalian, China) following the manufacturer's instructions. Quantitative real-time PCR was performed using SYBR® Fast qPCR Mix (TAKARA) in a total volume of 20 µl on 7900HT fast Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The relative expression of AFAP1-AS1 was normalized to GAPDH.

Cell culture

Four CCA cell lines (QBC939, CCLP1, HuCC-T1 and RBE), the nonmalignant cholangiocyte cell line BEC, and the human embryonic kidney cell line 293T were purchased from the Cell Resources Center of Shanghai Institutes for Biological Science, Chinese Academy of Science (Shanghai, China). All cells were grown in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Invitrogen, Grand Island, NY, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in an incubator with 5 % CO₂.

Vector construction and virus infection

In order to knockdown the AFAP1-AS1 expression in CCA cells, we inserted two different shRNA sequences targeting AFAP1-AS1 into pLKO.1-puro vector (Addgene, Cambridge, MA, USA) our previous

described. CCLP1 and RBE cells with high endogenous AFAP1-AS1 were selected for silencing. Lentivirus packaging, cell infection and selection of puromycin-resistant cells were performed as our previous described [19].

CCK-8 cell proliferation assay and clone formation assay

For proliferation assay, 3×10^3 CCLP1 and RBE cells were seeded on 96-well plates after lentivirus transfection. 10 μ L Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan) was added into each well at 24, 48, 72, and 96h, respectively. After an additional 2 h of incubation, the absorbance was measured at 450 nm using an ELISA reader (TECAN, Mannedorf, Switzerland).

For clone formation assay, 1×10^3 cells were plated in a 6-well plate with different cells and cultured for 14 days and colonies were then stained with 0.5% crystal violet, and the number of colonies more than 50 cells was counted following the established methods.

Cell cycle analyses by flow cytometry

Cells were harvested and fixed in 70% ethanol overnight at 4 °C. Then cells were stained with 25 μ g/ml propidium iodide (Sigma-Aldrich, St. Louis, MO, USA) containing 1 μ g/ml RNase (Sigma-Aldrich) at 37 °C for 30 min. The cells were analyzed for their distribution in different phases of cell cycle on FACScalibur flow cytometer using CellQuestPro software (Becton Dickinson, USA).

Cell migration and invasion assays

The effects of AFAP1-AS1 on cell migration and invasion were assessed using Transwell assays as our previous described [19]. In brief, 5×10^4 cells were seeded in triplicates onto a 24-well plate with Boyden chambers (BD Biosciences, San Jose, CA, USA) coated with Matrigel or without Matrigel. The lower chamber contained 10% FBS medium as chemoattractant. Cells were incubated for 36 h, stained with 0.5% crystal violet and counted under a microscope.

Tumorigenicity assays in nude mice

CCLP1 cells (1×10^7) transduced with SCR or AFAP1-AS1 shRNA#1 were suspended in 0.1 ml PBS and injected into left flank of 4-week-old female BALB/c athymic nude mice. Tumor volumes were calculated using a hand calipers every week after the injection using the following formula: tumor volume (mm³) = (length $\times$ width²)/2. At 6 weeks, mice were sacrificed and tumor volumes and weights were recorded. The experimental procedure involving animal tests was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and local institutional ethical guidelines, and the study was approved by the Experimental Animal Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

Western blot analysis

Western blot analysis was conducted as previously described [21]. In brief, cells were lysed ice-cold RIPA buffer (Promega, Madison, WI USA) containing proteinase inhibitor cocktail (Roche, Indianapolis, IN, USA). After blocking with 1% bovine serum albumin (BSA), the membranes were incubated with anti-c-Myc antibody, anti-Cyclin D1 antibody, anti-MMP-2 antibody, anti-MMP-9 antibody, and anti-GAPDH antibody, all from CST (Cell Signaling Technology, Danvers, MA, USA).

Statistical Analysis

Results are presented as means $\pm$ SD (standard deviation) of three independent experiments. Student's t-test was used to compare data between control and transfected cells. The Kaplan-Meier method was used to determine survival analysis. Chi-Square test was performed to evaluate the correlations between AFAP1-AS1 expression and clinicopathologic factors. P values of <0.05 were considered to be statistically significant. Data were analyzed using GraphPad Prism v.6 (La Jolla, CA, USA).

Results

Overexpression of AFAP1-AS1 in CCA tissues

Recent studies have shown that AFAP1-AS1 promoted cells proliferation and invasion in nasopharyngeal carcinoma, hepatocellular carcinoma, esophageal squamous cell carcinoma,

Fig. 1. LncRNA AFAP1-AS1 is significantly upregulated in CCA cell lines and tissues. (A) LncRNA AFAP1-AS1 abundance in nonmalignant cholangiocyte cell (BEC) and 4 CCA cell lines (QBC939, CCLP1, HuCC-T1 and RBE). (B) Relative expression level of lncRNA AFAP1-AS1 in 56 paired benign and malignant cholangiocyte tissues. (C) Relative expression level of lncRNA AFAP1-AS1 in stages I-II of CCA patients and stages III of CCA patients. **P < 0.01.

Fig. 2. Kaplan-Meier curves for overall survival in patients with intrahepatic cholangiocarcinoma according to AFAP1-AS1 expression. (A) The survival time of patients with high AFAP1-AS1 expression was significantly shorter than that in patients with low AFAP1-AS1 expression. (B) The survival time of patients with clinical stage III was significantly shorter than that in patients with clinical stage I-II.

gallbladder cancer [17, 19, 22, 23]. To investigate roles played by AFAP1-AS1 in CCA, we first examined the expression of AFAP1-AS1 in all 4 CCA cell lines (QBC939, CCLP1, HuCC-T1 and RBE) and the nonmalignant cholangiocyte cell line BEC. The expression of AFAP1-AS1 was considerably higher in CCA cells compared to BEC (Fig. 1A). Furthermore, 56 pairs of CCA tissues and corresponding adjacent normal bile duct tissues were used to detect the expression of AFAP1-AS1 and our results showed that AFAP1-AS1 was significantly increased in cancerous tissues (Fig. 1B). In addition, AFAP1-AS1 expression was significantly higher in advance stage lesions (stage III) than that in low stage I-II lesions (Fig. 1C). Together, these results suggested that AFAP1-AS1 might play a role in the development of CCA.

Table 1. Correlation between AFAP1-AS1 expression and the clinicopathological features in CCA patients. Abbreviations: TNM, tumor-node-metastasis

Variables	AFAP1-AS1 expression level		P-value
	Low N=28	High N=28	
Gender			
Female	4	5	1.000
Male	24	23	
Age, y			
≤55	7	9	0.7681
>55	21	19	
Liver cirrhosis			
No	6	10	0.3753
Yes	22	18	
Tumor size			
≤5cm	20	11	0.0306
>5cm	8	17	
Tumor number			
Single	21	23	0.7458
Multiple	7	5	
Tumor envelope			
Yes	17	14	0.5913
No	11	14	
HBsAg			
No	27	26	1.000
Yes	1	2	
TNM stage			
I / II	22	12	0.0129
III	6	16	
Vascular invasion			
No	23	13	0.0111
Yes	5	15	
Intrahepatic metastasis			
No	25	20	0.1771
Yes	3	8	

Abbreviations: TNM, tumor-node-metastasis;

Fig. 3. Knock-down of AFAP1-AS1 significantly inhibited cell proliferation *in vitro*. (A) AFAP1-AS1 expression in stable knockdown was confirmed by real time PCR. (B) CCK-8 assay was performed to measure proliferation in RBE and CCLP1 cells. (C) Cell clonogenicity was measured by the colony formation assay. (D) Cell cycle phase distributions were analyzed using flow cytometry. (E) Western blotting revealed that knock-down of AFAP1-AS1 decreased the levels of c-Myc and Cyclin D1. * $P < 0.05$, ** $P < 0.01$.

Relationship between AFAP1-AS1 expression and the clinicopathological features of patients with CCA

To further assess the association between the expression level of AFAP1-AS1 and clinicopathologic characteristics of patients with CCA, the 56 patients were divided into two groups according to the median expression of AFAP1-AS1 (Table 1). High AFAP1-AS1 expression in CCA had positive correlations with tumor size ($P=0.0306$), vascular invasion ($p=0.0129$), and advance TNM stage ($P=0.0111$). However, there was no significant association observed between AFAP1-AS1 levels and other clinicopathologic characteristics, such as age, patient gender, or HBV status. In addition, Kaplan-Meier analysis was used to evaluate the association between AFAP1-AS1 expression and 56 patients' survival. Our results showed that patients with high AFAP1-AS1 expression had significantly shorter overall survival time than those with low AFAP1-AS1 expression (Fig. 2A). Interestingly, according to the survival curves, patients in TNM stage I-II had shorter overall survival time than those in TNM stage III (Fig. 2B). Taken together, our results demonstrated that the expression of AFAP1-AS1 was significantly associated with clinical characteristics and poor overall survival.

Fig. 4. Silencing of AFAP1-AS1 inhibited cell migration and invasion in CCA cells. (A) Representative pictures of migratory and invasive RBE cells were stained and the average number of cells was calculated. (B) Representative pictures of migratory and invasive CCLP1 cells were stained and the average number of cells was calculated. (C) Western blotting revealed that downregulated MMP-2 and MMP-9 levels in AFAP1-AS1 knockdown CCA cells. ** $P < 0.01$.

AFAP1-AS1 silencing markedly decreased the proliferation and arrested cell cycle of cholangiocarcinoma cells in vitro

Since overexpression of AFAP1-AS1 was found in CCA tissues compared to non-carcinoma tissues and associated with tumor size. So it was interesting to examine roles of AFAP1-AS1 in cell proliferation. Knockdown AFAP1-AS1 using lentiviral and the AFAP1-AS1 expression was detected to confirm the efficiency of silencing (Fig. 3A). Next, we assessed the effect of AFAP1-AS1 silencing on cell proliferation, colony formation, and cell cycle. As shown in figure 3B, AFAP1-AS1 knockdown inhibited cell growth of both CCA cell lines (Fig. 3B). Further, the number and size of the colonies formed were markedly decreased in the AFAP1-AS1 knockdown cells (Fig 3C). To investigate the mechanisms underlying altered cell growth, flow cytometry assay was performed to examine the regulatory effect of AFAP1-AS1 on the distribution of cell cycle. We found that treatment of AFAP1-AS-specific knockdown resulted in a smaller part of the population in S phase and an enhanced proportion in G0-G1phase (Fig. 3D). In addition, suppression of AFAP1-AS1 impaired the expression of c-Myc and Cyclin D1 (Fig. 3E). The above results suggested that AFAP1-AS1 regulated proliferation in CCA cells.

Inhibition of AFAP1-AS1 inhibited cell migration and invasion by decreasing MMP-2 and MMP-9 expression

Given that AFAP1-AS1 associated with vascular invasion in CCA specimens, we studied whether vascular invasion could affect CCA cells migration and invasion. A directional migration was examined using a transwell assay and our results showed that the migration of CCA cells was notably decreased when AFAP1-AS1 knockdown (Fig. 4A and B). Furthermore, matrigel invasion assays also demonstrated that knockdown of AFAP1-AS1 could attenuate the invasive ability of both CCA cells (Fig. 4A and B). In addition, knockdown of endogenous AFAP1-AS1 expression could decrease MMP-2 and MMP-9 expression compared to the SCR group (Fig. 4C). However, the altered expression level of AFAP1 was not observed (Fig. 4C). These results indicated that AFAP1-AS1 regulated cell invasion by altering the MMP-2 and MMP-9 expression.

Fig. 5. Knockdown of AFAP1-AS1 suppressed tumor growth *in vivo*. (A) Photographs of the xenograft tumor masses of CCLP1 transduced with SCR or AFAP1-AS1 siRNA. (B) Tumor volumes were measured weekly from week one to six post-injection. (C) Tumor weights were measured after mice sacrificed. (D) The expression of c-Myc in xenograft tumor was detected by western blotting. ** $P < 0.01$.

AFAP1-AS1 downregulation repressed tumor growth in a mice model

Based on the observed inhibition of migratory, invasive and proliferative behaviors in CCA cells transduced with AFAP1-AS1 siRNA, we next confirm the effect of AFAP1-AS1 on CCLP1 *in vivo*. As shown in Figure 5A, 6 weeks after CCLP1 cell subcutaneous inoculation, lentivirus mediated AFAP1-AS1 knockdown can significantly suppress the growth of CCA xenografts in nude mice. The average tumor weight in the AFAP1-AS1 silencing group was significantly lower than that in the SCR group (Fig. 5B). Tumor growth curves also showed that the volume of tumors formed by AFAP1-AS1 silencing CCLP1 cells were significantly smaller than that formed by SCR cells (Fig. 5C). In addition, western blot analysis indicated that knockdown of AFAP1-AS1 led to a decrease of c-Myc expression (Fig. 5D). These results suggested that AFAP1-AS1 silencing suppressed tumor growth *in vivo* and could serve as a therapeutic target in CCA therapy.

Discussion

In recent years, although thousands of lncRNAs have recently been identified, the roles in modulating gene expression were still unknown. In our present study, we found that AFAP1-AS1 acted as an oncogene of cholangiocarcinoma growth both *in vitro* and *in vivo*, suggesting a therapeutic potential for CCA patients in the future.

Previous studies have shown that lncRNA AFAP1-AS1 was upregulated in NSCLC tissues and patients with high AFAP1-AS1 expression had shorter overall survival [24]. Similar results were also found in esophageal adenocarcinoma, hepatocellular carcinoma, and pancreatic ductal adenocarcinoma [18-20]. Consisted with previous study report, we found that AFAP1-AS1 expression was markedly decreased in CCA tissues compared with adjacent non-tumor tissues. Furthermore, high HNF1A-AS1 expression was associated with tumor size, vascular invasion, clinical stage, and short overall survival rates of CCA patients. It is reported that AFAP1-AS1 could regulate tumor proliferation, invasion, and apoptosis in esophageal adenocarcinoma, hepatocellular carcinoma, and pancreatic ductal adenocarcinoma [18-20]. AFAP1-AS1 was first reported to increase and hypomethylate in esophageal adenocarcinoma and inhibition of AFAP1-AS1 significantly increased apoptosis of EAC cell and induced G2/M-phase arrest [18]. In lined with the results, our experiments also demonstrated that AFAP1-AS1 knockdown resulted in suppression of proliferation via arresting cell cycle. Cyclin D1 and c-Myc were found to be deregulated and acted as proto-oncogene and good biomarker for

tumor progression in several cancers, including CCA [25-27]. As expected, our results showed that the protein levels of Cyclin D1 and c-Myc were decreased in AFAP1-AS1 knockdown cells. Furthermore, it was recently reported ectopic AFAP1-AS1 expression promoted cell migration and invasion by increasing the mesenchymal marker (Slug, N-cadherin, and vimentin), while epithelial marker (E-cadherin) was increased at the same time [20]. It is well known that aberrant MMP-2 and MMP-9 expression was involved in metastasis [28]. As we expected, the findings of our study showed that AFAP1-AS1 silencing resulted in a significant decrease in cell migration and invasion with downregulation of MMP-2 and MMP-9, which was consistent with others reports [23, 29].

In conclusion, this study provided the first evidence that increased expression of lncRNA AFAP1-AS1 was associated with the malignant behavior and poor prognosis in CCA. The effects of AFAP1-AS1 on cell proliferation, migration, invasion, and cell cycle suggested that AFAP1-AS1 served as a potential target for CCA.

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Disclosure Statement

The authors declare no conflicts of interest.

References

- 1 Rizvi S, Gores GJ: Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology* 2013;145:1215-1229.
- 2 Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J: Cancer statistics in China, 2015. *CA Cancer J Clin* 2016;66:115-132.
- 3 Sirica AE, Nathanson MH, Gores GJ, Larusso NF: Pathobiology of biliary epithelia and cholangiocarcinoma: Proceedings of the Henry M. And Lillian Stratton Basic Research Single-Topic Conference. *Hepatology* 2008;48:2040-2046.
- 4 Li L, Che L, Tharp KM, Park HM, Pilo MG, Cao D, Cigliano A, Latte G, Xu Z, Ribback S, Dombrowski F, Evert M, Gores GJ, Stahl A, Calvisi DF, Chen X: Differential requirement for de novo lipogenesis in cholangiocarcinoma and hepatocellular carcinoma of mice and humans. *Hepatology* 2016;63:1900-1913.
- 5 Shin HR, Oh JK, Masuyer E, Curado MP, Bouvard V, Fang YY, Wiangnon S, Sripa B, Hong ST: Epidemiology of cholangiocarcinoma: An update focusing on risk factors. *Cancer Sci* 2010;101:579-585.
- 6 Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar SR, Bornstein JD, Kitrinos KM, Subramanian GM, McHutchison JG, Heathcote EJ: Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: A 5-year open-label follow-up study. *Lancet* 2013;381:468-475.
- 7 El-Serag HB, Kanwal F: Epidemiology of hepatocellular carcinoma in the United States: Where are we? Where do we go? *Hepatology* 2014;60:1767-1775.
- 8 Evans JR, Feng FY, Chinnaiyan AM: The bright side of dark matter: LncRNAs in cancer. *J Clin Invest* 2016;126:2775-2782.
- 9 Quinn JJ, Chang HY: Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet* 2016;17:47-62.
- 10 Pan Y, Li C, Chen J, Zhang K, Chu X, Wang R, Chen L: The emerging roles of long noncoding RNA ROR (lncRNA-ROR) and its possible mechanisms in human cancers. *Cell Physiol Biochem* 2016;40:219-229.
- 11 Zhou X, Ye F, Yin C, Zhuang Y, Yue G, Zhang G: The interaction between MiR-141 and lncRNA-H19 in regulating cell proliferation and migration in gastric cancer. *Cell Physiol Biochem* 2015;36:1440-1452.

- 12 Hu HB, Jie HY, Zheng XX: Three circulating lncRNA predict early progress of esophageal squamous cell carcinoma. *Cell Physiol Biochem* 2016;40:117-125.
- 13 Wang F, Yang H, Deng Z, Su Y, Fang Q, Yin Z: HOX antisense lincRNA HOXA-AS2 promotes tumorigenesis of hepatocellular carcinoma. *Cell Physiol Biochem* 2016;40:287-296.
- 14 Zhou X, Ye F, Yin C, Zhuang Y, Yue G, Zhang G: The interaction between MiR-141 and lncRNA-H19 in regulating cell proliferation and migration in gastric cancer. *Cell Physiol Biochem* 2015;36:1440-1452.
- 15 Wang WT, Ye H, Wei PP, Han BW, He B, Chen ZH, Chen YQ: LncRNAs H19 and HULC, activated by oxidative stress, promote cell migration and invasion in cholangiocarcinoma through a ceRNA manner. *J Hematol Oncol* 2016;9:117.
- 16 Ma SL, Li AJ, Hu ZY, Shang FS, Wu MC: Coexpression of the carbamoylphosphate synthase 1 gene and its long noncoding RNA correlates with poor prognosis of patients with intrahepatic cholangiocarcinoma. *Mol Med Rep* 2015;12:7915-7926.
- 17 Bo H, Gong Z, Zhang W, Li X, Zeng Y, Liao Q, Chen P, Shi L, Lian Y, Jing Y, Tang K, Li Z, Zhou Y, Zhou M, Xiang B, Li X, Yang J, Xiong W, Li G, Zeng Z: Upregulated long non-coding RNA AFAP1-AS1 expression is associated with progression and poor prognosis of nasopharyngeal carcinoma. *Oncotarget* 2015;6:20404-20418.
- 18 Wu W, Bhagat TD, Yang X, Song JH, Cheng Y, Agarwal R, Abraham JM, Ibrahim S, Bartenstein M, Hussain Z, Suzuki M, Yu Y, Chen W, Eng C, Grealley J, Verma A, Meltzer SJ: Hypomethylation of noncoding DNA regions and overexpression of the long noncoding RNA, AFAP1-AS1, in Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterology* 2013;144:956-966.
- 19 Lu X, Zhou C, Li R, Liang Z, Zhai W, Zhao L, Zhang S: Critical role for the long non-coding RNA AFAP1-AS1 in the proliferation and metastasis of hepatocellular carcinoma. *Tumour Biol* 2016;37:9699-9707.
- 20 Ye Y, Chen J, Zhou Y, Fu Z, Zhou Q, Wang Y, Gao W, Zheng S, Zhao X, Chen T, Chen R: High expression of AFAP1-AS1 is associated with poor survival and short-term recurrence in pancreatic ductal adenocarcinoma. *J Transl Med* 2015;13:137.
- 21 Zhu X, Li D, Yu F, Jia C, Xie J, Ma Y, Fan S, Cai H, Luo Q, Lv Z, Fan L: MiR-194 inhibits the proliferation, invasion, migration, and enhances the chemosensitivity of non-small cell lung cancer cells by targeting forkhead box A1 protein. *Oncotarget* 2016;7:13139-13152.
- 22 Luo HL, Huang MD, Guo JN, Fan RH, Xia XT, He JD, Chen XF: AFAP1-AS1 is upregulated and promotes esophageal squamous cell carcinoma cell proliferation and inhibits cell apoptosis. *Cancer Med* 2016;5:2879-2885.
- 23 Ma F, Wang SH, Cai Q, Zhang MD, Yang Y, Ding J: Overexpression of LncRNA AFAP1-AS1 predicts poor prognosis and promotes cells proliferation and invasion in gallbladder cancer. *Biomed Pharmacother* 2016;84:1249-1255.
- 24 Deng J, Liang Y, Liu C, He S, Wang S: The up-regulation of long non-coding RNA AFAP1-AS1 is associated with the poor prognosis of NSCLC patients. *BIOMED Pharmacother* 2015;75:8-11.
- 25 Wang Z, Tang X, Zhang Y, Qi R, Li Z, Zhang K, Liu Z, Yang X: Lobaplatin induces apoptosis and arrests cell cycle progression in human cholangiocarcinoma cell line RBE. *Biomed Pharmacother* 2012;66:161-166.
- 26 Sugimachi K, Aishima S, Taguchi K, Tanaka S, Shimada M, Kajiyama K, Sugimachi K, Tsuneyoshi M: The role of overexpression and gene amplification of cyclin D1 in intrahepatic cholangiocarcinoma. *J Hepatol* 2001;35:74-79.
- 27 Zhu X, Li D, Yu F, Jia C, Xie J, Ma Y, Fan S, Cai H, Luo Q, Lv Z, Fan L: MiR-194 inhibits the proliferation, invasion, migration, and enhances the chemosensitivity of non-small cell lung cancer cells by targeting forkhead box A1 protein. *Oncotarget* 2016;7:13139-13152.
- 28 Morton SD, Cadamuro M, Brivio S, Vismara M, Stecca T, Massani M, Bassi N, Furlanetto A, Joplin RE, Floreani A, Fabris L, Strazzabosco M: Leukemia inhibitory factor protects cholangiocarcinoma cells from drug-induced apoptosis via a PI3K/AKT-dependent Mcl-1 activation. *Oncotarget* 2015;6:26052-26064.
- 29 Han X, Wang L, Ning Y, Li S, Wang Z: Long non-coding RNA AFAP1-AS1 facilitates tumor growth and promotes metastasis in colorectal cancer. *Biol Res* 2016;49:36.