Original Paper

Elevated FOXC2 Expression Promotes Invasion of HCC Cell Lines and is Associated with Poor Prognosis in Hepatocellular Carcinoma

Fang Yang Lizhi Lv Kun Zhang Qiucheng Cai Jianyong Liu Yi Jiang

Department of Hepatobiliary Surgery, Fuzong Clinical Medical College, Fujian Medical University, Fuzhou, Fujian, China

Key Words

Foxc2 • HCC • Proliferation • Invasion • Prognosis

Abstract

Background/Aims: Increasing evidence has indicated that Forkhead box protein C2 (FOXC2) plays an important role in carcinogenesis. However, the expression and the role of FOXC2 in hepatocellular carcinoma (HCC) have not been extensively studied. **Methods:** FOXC2 expression was analyzed by quantitative real-time polymerase chain reaction, Western blot analysis and immunohistochemistry in HCC tissue and cells. The relationship between FOXC2 expression and patient clinical significance and survival were assessed by Pearson's correlation and Kaplan-Meier analysis, respectively. Cell proliferation assays, colony formation assays, flow cytometric analysis and Transwell assays were employed to measure the effects of FOXC2 on HCC cells *in vitro*. **Results:** The expression of FOXC2 was increased in HCC tissue, and high FOXC2 expression was associated with worse patient survival. Knockdown of FOXC2 inhibited HCC cell growth, migration, and invasion *in vitro*, as well as tumor growth. Furthermore, we found that activation of AKT-mediated MMP-2 and MMP-9 was involved in FOXC2 promoting an aggressive phenotype. **Conclusions:** Taken together, these findings demonstrate that FOXC2 is upregulated in HCC tissue and is associated with tumor size, vascular invasion and advanced TNM stage. Further investigation suggested that FOXC2 may play a vital role in promoting proliferation and invasion in HCC and serves as a novel therapeutic target in HCC.

Introduction

Human hepatocellular carcinoma (HCC) is the fifth most common malignant tumor and the third most prevalent cause of cancer-related mortality globally, especially in East Asia and sub-Saharan Africa [1-3]. In spite of major improvements in early detection, liver surgical

© 2017 The Author(s)
Published by S. Karger AG, Basel

Yi Jiang

Department of Hepatobiliary Surgery, Fuzong Clinical Medical College,
Fujian Medical University, No. 156 West Second Ring Road, Fuzhou, (China)
Tel. +86-591-22859377, E-Mail jiangyi135@163.com

KARGER

resection, and adjuvant therapy, HCC patients still experience high rates of recurrence and poor prognosis, mainly due to the high invasion and metastatic capacities of these cancer cells [4-7]. Molecular studies have revealed a large number of genetic alterations that lead to HCC; however, precise mechanisms responsible for the occurrence and progression of HCC still remain poorly understood [8-10]. Therefore, it is critical to elucidate the mechanisms underlying prognosis prediction as well as novel therapeutic strategies.

Forkhead box C2 (FOXC2), a member of the FOX transcription factor superfamily of proteins, was first identified in mice during embryogenesis [11]. Accumulating evidence has shown that FOXC2 is involved in tumor cell proliferation, invasion, differentiation, and cisplatin-resistance [12-15]. In extrahepatic cholangiocarcinoma, FOXC2 acts as an oncogene by regulating the ability to migrate and invade with concomitant upregulation of N-cadherin, MMP-2 and Ang-2, and high FOXC2 expression is associated with a shorter duration of recurrence-free survival and poor prognosis [13]. In colorectal cancer, FOXC2 was found to be upregulated in colorectal cancer tissues and high FOXC2 expression was correlated with aggressive phenotypes and poor survival of patients with colon cancer [14]. FOXC2 was reported to be upregulated in CDDP-resistant ovarian cancer cells and knockdown of FOXC2 could reduce the capacity of migration, invasion, and attachment of a CDDP-resistant ovarian cancer cell reverse epithelial-mesenchymal transition (EMT) phenotype [15]. However, whether FOXC2 participates in the malignant phenotype in HCC remains unclear and needs to be further explored.

In this study, we investigated the expression pattern and potential role of FOXC2 in the progression of HCC. Our results suggest that FOXC2 expression is significantly upregulated in HCC tissue when compared with matched adjacent normal tissues, and high expression levels of FOXC2 is associated with worse outcomes in HCC patients. Furthermore, we also explored the role of FOXC2 in HCC cell biological functions and the underlying molecular mechanisms.

Materials and Methods

Tissue samples

A total of 84 tumor tissues and matched adjacent tissues were collected from patients undergoing surgical resection at the Department of Hepatobiliary Surgery, Fuzong Clinical Medical College of Fujian Medical University, between Oct 2008 and May 2011. All HCC patients were histologically diagnosed by staining with hematoxylin and eosin (H and E) by two independent histopathologists. None received any anti-tumor treatment prior to surgery. Written informed consent was obtained from all enrolled patients, and the study was approved by the ethics committee of Fujian Medical University.

Immunohistochemical staining (IHC)

IHC staining was performed using a standard streptavidin-biotin-peroxidase complex method as previously described [16]. In brief, paraffin sections were deparaffinized and hydrated. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H₂O₂) for 15 min. Antigen was retrieved by microwaving sections for 10 min in 10 mM citrate buffer (pH 6.0). Next, tumor sections were incubated with FOXC2 primary antibody (1:100, Cell Signaling Technology, Boston, USA) at 4°C overnight in a humidified chamber. After washing, the slides were incubated with horseradish peroxidase-conjugated anti-goat antibody (DakoCytomation, Carpinteria, CA, USA) at room temperature for 30 min. 3, 5-diaminobenzidine (DAB) substrate was used to visualize color development followed by Mayer's hematoxylin counterstaining. Negative control slides that omitted the primary antibodies were included in all assays.

For the IHC score, the expression level was independently assessed by two independent pathologists, according to the proportion and intensity of positive cells. The intensity of staining was scored from 0 to 3, representing negative (-), weak (+), moderate (++) and strong (+++) staining, and the extent of staining was scored as 0 (0-25%), 1 (26-50%), 2 (51-75%), and 3 (76-100%). The final quantitation of each sample was obtained by multiplying the two scores.

Cell culture

The human HCC cell lines (SMMC-7721, HepG2, Hep3B, and MHCC-97H), and an immortalized hepatic epithelial cell line, LO2, were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and routinely maintained in high-glucose DMEM (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Life Technologies, Grand Island, NY, USA) in a humidified incubator at 37°C under 5% CO₂.

RNA isolation and quantitative real-time PCR

Total RNA was extracted from HCC and adjacent tissues or cells using TRIzol (Invitrogen) following the manufacturer's instructions. cDNA was then reverse-transcribed from 500 ng of RNA using a Prime Script™ RT-PCR kit (Takara Biochemicals, Tokyo, Japan) on an ABI-9700 (Applied Biosystems). Quantitative real time PCR was performed using SYBR® Premix Ex Taq™ II (Takara) in an ABI PRISM®7900HT Real-Time PCR System. The primers used for qRT-PCR detection included human FOXC2 forward, 5' -CCTCCTGGTATCTCAACCACA-3', and reverse, 5' -GAGGGTCGAGTTCTCAATCCC-3'. Human GAPDH, forward, 5' -GGTGAAGGTCGGAGTCAACG-3', and reverse, 5' -ACCATGTAGTTGAGGTCAATGAAGG-3'. Relative mRNA expression levels were calculated by the 2^{-ΔΔCt} rate method and were normalized to the internal control of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH was used as internal control.

Construction of vectors and stable transfection of HCC cells

The short hairpin RNA (shRNA) sequences targeting FOXC2 were designed and constructed by GenePharma Co.,Ltd (Shanghai, China). The shRNA target sequence of human FOXC2 was subcloned into the lentiviral vector pLKO.1-TRC cloning vector (Sigma, St Louis, MO, USA). A PLKO.1-scramble (SCR) with limited homology with any known sequences in humans was used as a negative control. Hep3B and MHCC-97H cells were transfected with the shFOXC2 vector or SCR. The stably transfected cells were selected using 2 μg/ml puromycin (Sigma) 48 h later.

CCK-8 cell viability assay and colony formation assay

For the cell viability assay, cells were plated into a 96-well plate at 3×10³ cells per well in triplicate with 100 μl cultured medium. Ten μl of cell counting kit-8 (CCK8, Dojindo) was added into each well at 24, 48, 72, and 96 h. After 2 h incubation, the absorbance of cells was measured at 450 nm using a microplate reader (Bio-Rad, 3550, Hercules, CA, United States).

For the colony formation assay, stably transfected cells were seeded onto 6-well plates at 1×10³ cells per well in triplicate. The media was replaced every 3 days. Two weeks later, colonies were fixed with 100% methanol for 15 min and stained with 0.5% crystal violet for 30 min. The colonies numbering more than 50 cells were considered to be clones and counted.

Flow cytometry assays

The transfected HCC cells were washed twice with PBS and harvested. Then, cells were stained with 5 μl of Annexin V-FITC and with 5 μl of PI solution (BD Biosciences, San Jose, CA, USA) for 15 min at room temperature. Flow cytometric analysis was performed using a two-color fluorescence-activated cell sorting (FACS) analyzer (Beckman Coulter, cytomics FC 500, CA, USA) to detect apoptosis.

Cell migration and invasion assay

A total of 4 × 10⁴ various cells in 100 μl serum-free DMEM medium were plated into the upper chamber of an 8 μm pore membrane (Costar, Corning, NY, USA) coated with Matrigel (BD Matrigel™ matrix; BD Bioscience, Heidelberg, Germany) (invasion) or without Matrigel (migration) and to the lower chamber, 600 μl complete medium was added as a chemoattractant. After incubation for 36 h, the non-invading cells were scraped with a cotton swab and the membranes were then fixed with 100% methanol and stained with 0.5% crystal violet. The membranes were photographed using a digital light microscope and five fields were selected randomly to obtain the mean cell number on each membrane.

Western blot analysis

Protein was extracted from cultured cells using RIPA lysis buffer (Cell Signaling Technology, Danvers, MA, USA) supplemented with protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). Identical quantities of proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membrane (Bio-Rad). After blocking with 5% skim milk for 2 h at room temperature, the membranes were incubated with antibody overnight at 4°C. The antibodies

against FOXC2, BCL-2, Cleaved PARP (Asp214), Cleaved Caspase-3, AKT, p-AKT, MMP-2, MMP-9 and GAPDH were purchased from Cell Signaling Technology. The protein bands were visualized using SuperSignal West Femto Chemiluminescent Substrate (Thermo Scientific™ Pierce, Appleton, WI, USA) according to the manufacturer's instructions.

Animal experiments

Six-week old NOD/SCID female mice were purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai). Thirty-two nude mice were randomized into four groups of 8 nude mice each. All animal experiments were approved by the institutional animal care and use committee of Fujian Medical University. All procedures were in accordance with the animal care and use committee of the institution and conformed to legal mandates and national guidelines for the care and maintenance of laboratory animals. 1×10^7 Hep3B cells stably expressing control vector or FOXC2 shRNA were subcutaneously injected into the left flank of SCID mice. The size of the tumor was monitored every week using a caliper along two perpendicular axes. After 5 weeks following cell injection, mice were sacrificed, and tumors were excised, weighed, and subjected to IHC staining.

Statistical analysis

All statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL, USA). The Student's t-test was used to compare the differences between experimental and control groups. The correlation between FOXC2 expression and the clinicopathological parameters was evaluated using the Pearson chi-square test. Survival plots were analyzed by Kaplan-Meier analysis, and the log-rank test was used to assess the significance of the differences. Differences were defined as statistically significant for p-values < 0.05.

Results

Aberrant overexpression of FOXC2 in HCC tissues and cell lines

To characterize the role of FOXC2 in HCC, we analyzed the expression of FOXC2 in 84 paired HCC tissues and corresponding adjacent tissues using qRT-PCR. Our results showed that the mRNA expression of FOXC2 was significantly upregulated in HCC tissues compared with paired normal tissues (Fig. 1A). Interestingly, the FOXC2 mRNA expression level was markedly correlated with the TNM stage of HCC patients (Fig. 1B). Furthermore, compared with an immortalized hepatic epithelial cell line LO2, the protein expression of FOXC2 was markedly increased in all four HCC cell lines (SMMC-7721, HepG2, Hep3B, and MHCC-97H) (Fig. 1C). These results indicate that increased FOXC2 expression may be critically involved in HCC progression.

High FOXC2 expression is correlated with poor prognosis in HCC

To determine whether the protein level of FOXC2 changed in HCC tissues, we performed IHC assays to further analyze the expression of FOXC2 in HCC tissues and paired adjacent tissues. The results revealed that the protein level of FOXC2 was more commonly upregulated in HCC tissues compared with normal counterparts (Fig. 2A). The relationships between FOXC2 expression in the HCC samples and the patients' ages, gender, tumor size, tumor number, HBsAg, Cirrhosis, serum AFP, venous invasion, and TMN stages are shown in Table 1. High FOXC2 expression was significantly associated with larger tumor size ($P = 0.0065$), vascular invasion ($P = 0.0093$), and TNM stage ($P = 0.0003$). However, there was no significant correlation between FOXC2 expression level and other clinicopathological features. Furthermore, Kaplan-Meier survival analysis revealed that HCC patients with high FOXC2 expression had a shorter overall survival time than those in the low FOXC2 expression group (Fig. 2B). In addition, the overall survival rate of the TNM stage III group was significantly worse than that of the TNM stage I - II group (Fig. 2C). These observations suggest that increased expression of FOXC2 is associated with the progression and development of HCC.

FOXC2 silencing inhibits proliferation and growth of HCC cells in vitro

The significantly increased expression of FOXC2 in HCC tissues prompted us to explore its biological role in HCC cells. Following knockdown of the expression of FOXC2 with len-

Fig. 1. Evaluation of FOXC2 expression in primary human HCC tissues and HCC cell lines. (A) Analyses of FOXC2 mRNA expression in 84 pairs of HCC samples and adjacent normal liver tissue. (B) mRNA expression of FOXC2 in TNM I - II stage and TNM III stage patients by qRT-PCR. (C) Western blot analysis was performed to assess FOXC2 protein levels in HCC cell lines and LO2 cells. ** $P < 0.01$.

Fig. 2. FOXC2 overexpression is associated with aggressive HCC. (A) Representative IHC staining of FOXC2 in adjacent normal tissues (left) and HCC tissues (right) (IHC $\times 200$). (B) Kaplan-Meier curve indicating that patients with high expression of FOXC2 have a worse overall survival compared to patients with low expression of FOXC2 ($P=0.0063$). (C) Kaplan-Meier analyses indicating that patients in TNM III stage had a shorter overall survival ($P=0.0048$).

tiviral shRNA transduction of MHCC-97H and Hep3B cells, Western blotting was performed to confirm the successful knockdown of FOXC2 in these two cell lines (Fig. 3A). The CCK-8 assay results indicated that knockdown of FOXC2 significantly reduced MHCC-97H and Hep3B cell viability compared with scramble controls (Fig. 3B). Apoptosis analysis was used to assess the role of FOXC2 in HCC cell apoptosis and showed that FOXC2 depletion led to a significant increase in apoptosis (Fig. 3C). In addition, colony formation assays indicated that FOXC2 silencing obviously decreased the colony formation ability of MHCC-97H and Hep3B cells compared with scramble cells (Fig. 3D). These results suggest that FOXC2 plays an important role in HCC cell proliferation.

Knockdown of FOXC2 impairs HCC cell migration and invasion

We continued to explore the effects of FOXC2 on HCC invasion *in vitro*. Transwell assays demonstrated that cell migration and invasion were significantly suppressed in HCC cells transduced with shFOXC2 compared with cells transfected with scramble (Fig. 4A and B). To evaluate the effects of FOXC2 knockdown on HCC cell proliferation and invasion, the expression of proliferation-related genes (p-AKT), apoptosis-related genes (cleaved PARP, cleaved Caspase-3 and BCL-2), and invasion-related genes (MMP-2 and MMP-9) were detected by Western blotting analysis. The depletion of FOXC2 significantly decreased the expression of BCL-2, p-AKT, MMP-2, and MMP-9 in MHCC-97H and Hep3B cells, whereas the expression levels of cleaved PARP and cleaved Caspase-3 were increased (Fig. 4C). However, the expression level of total AKT was unaltered (Fig. 4C). These results suggest that FOXC2 promotes HCC cell migration and invasion.

Stable silencing of FOXC2 reduces tumor growth in a xenograft model

We further investigated the effect of FOXC2 knockdown on HCC tumor growth *in vivo*. Hep3B cells transduced with scramble or shFOXC2 were subcutaneously injected into the flanks of nude mice. As shown in Fig. 5A, for tumors from Hep3B with knocked-down FOXC2, tumor size was significantly smaller than in the scramble group. Both tumor weight speed and tumor growth were also significantly inhibited when FOXC2 was knocked down (Fig. 5B and C). In addition, we performed IHC to determine whether knockdown of FOXC2 can reduce the expression of Ki-67 in tissues taken from tumors in nude mice. Consistent with our *in vitro* results, knockdown of FOXC2 markedly decreased the protein expression level of

Table 1. Correlation between FOXC2 expression and clinicopathological characteristics of HCC patients

Characteristics	FOXC2		P value
	High cases (n=42)	Low cases (n=42)	
Age (years)			0.4949
≤50	13	7	
> 50	29	5	
Gender			1
Male	35	6	
Female	7		
HBsAg			1
Yes	39	0	
No	3		
Cirrhosis			0.2663
Yes	28	2	
No	14	0	
Serum AFP (μg/L)			0.3828
≤20	18	3	
>20	24	9	
Tumor size			0.0079
≤5 cm	12	5	
>5 cm	30	7	
Tumor number			0.4949
Single	25	9	
Multiple	17	3	
Vascular invasion			0.0116
Yes	21		
No	21	3	
TNM Stage			0.0003
I-II	16	3	
III	26

Fig. 3. Knockdown of FOXC2 expression inhibits HCC cell growth in vitro. (A) The protein level of FOXC2 was markedly reduced in MHCC-97H and Hep3B after silencing. (B) FOXC2 silencing significantly inhibits cell viability both in MHCC-97H and Hep3B cells. (C) Knockdown of FOXC2 induces cell apoptosis as measured by flow cytometry both in MHCC-97H and Hep3B cells. (D) Knockdown of FOXC2 inhibited colony formation as demonstrated by colony formation assay in MHCC-97H and Hep3B cells. ** $P < 0.01$.

FOXC2, and Ki-67 was found to be much lower in tumors with FOXC2 knockdown compared with tumors with scramble (Fig. 5D). These results suggest that knockdown of FOXC2 could suppress tumor growth *in vivo*.

Fig. 4. Effects of FOXC2 silencing on invasion ability of HCC cell lines. (A) Representative images and quantification of the effects of FOXC2 silencing on the migratory and invasive abilities of MHCC-97H cells as determined by Transwell assays. (B) Representative images and quantification of the effects of FOXC2 silencing on the migratory and invasive abilities of Hep3B cells as determined by Transwell assays. (C) Western blot analysis showing downregulation of BCL-2, cleaved PARP, cleaved Caspase-3, p-AKT, MMP-2 and MMP-9 in FOXC2 and upregulation of cleaved PARP and cleaved Caspase-3 in FOXC2-depleted MHCC-97H and Hep3B cells. **P < 0.01.

Discussion

Recent studies have shown that FOXC2 plays a causal role in the regulation of diverse biological actions, including cell growth, apoptosis, tumor metastasis and angiogenesis [13,

Fig. 5. Knockdown of FOXC2 inhibits tumorigenesis of HCC cells. (A) Representative picture of tumor formation in the Hep3B scramble group or FOXC2-depleted Hep3B group in nude mice. (B) Tumor volumes were measured every week. (C) Tumor weight was decreased in the FOXC2-depleted Hep3B group mouse model. (D) Representation of FOXC2 and Ki-67 expression in xenograft tumors using IHC in the Hep3B scramble group or the FOXC2-depleted Hep3B group. ** $P < 0.01$.

14, 17]. In our present study, we demonstrated a critical role of FOXC2 in regulating the progression of HCC. A higher expression level of FOXC2 was observed in HCC tissues and cells and elevated expression of FOXC2 was positively associated with tumor size, vascular invasion, advanced TNM stage, and poor patient survival. Knockdown of FOXC2 expression decreased cell growth, colony formation, migration, and invasion and induced apoptosis in HCC cells *in vitro*. The effects of FOXC2 on tumor progression may be mediated through the altered expression of p-AKT, MMP-2 and MMP-9. In addition, FOXC2 silencing inhibited tumorigenesis *in vivo*. To the best of our knowledge, this is the first study exploring the relationship between FOXC2 and HCC progression, and our findings indicate that FOXC2 may serve as a novel therapeutic target in HCC patients.

Despite previous studies that have demonstrated that FOXC2 has a major role in the progression of tumors, its role in tumor growth and metastasis appears to be more complex. The overexpression of FOXC2 has been found to be correlated with poor prognosis of patients and promotes proliferation, EMT, and metastasis via AKT or ERK/GSK-3 β signaling in colorectal cancer [14, 18]. FOXC2 has been reported to play a crucial role in invasion, metastasis, EMT, and stem cell-like properties in breast cancer [17, 19, 20]. Additional studies have shown a starkly inverse association between FOXC2 and E-cadherin expression in non-small cell lung cancer and squamous cell carcinoma. The molecular mechanism behind this correlation was shown to be that FOXC2 downregulates p120-catenin, which

directly suppresses the promoter activity of E-cadherin [21]. FOXC2 has been reported to enhance AKT activity to promote cell proliferation and invasion [13, 22, 23]. In our study, we found that FOXC2 is upregulated in HCC tissues and that high FOXC2 expression is correlated with poor prognosis, suggesting that FOXC2 contributes to the acquisition of the aggressive properties of HCC cells. Further study found that shRNA-mediated FOXC2 silencing inhibited cell proliferation and reduced the expression of Ki-67 *in vitro* and *in vivo*.

In conclusion, this is the first report to describe that FOXC2 acts as an oncogene in HCC. High FOXC2 expression correlates with poor overall survival, vascular invasion, and advanced TNM stage. Further studies assessed the biological function of FOXC2 on HCC cells *in vitro* and *in vivo*, suggesting that inhibition of FOXC2 expression in HCC is a promising molecular target for patients with HCC.

Disclosure Statement

The authors of this manuscript have no conflicts of interest.

Acknowledgements

This work was supported by the Innovation Team Funds of Fuzhou General Hospital of Chinese PLA (No. 2014cxt005) and the Natural Science Foundation of Fujian province (No. 2015J01489).

References

- 1 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: Global cancer statistics, 2012. *CA Cancer J Clin* 2015;65:87-108.
- 2 Deng B, Qu L, Li J, Fang J, Yang S, Cao Z, Mei Z, Sun X: MiRNA-211 suppresses cell proliferation, migration and invasion by targeting SPARC in human hepatocellular carcinoma. *Sci Rep* 2016;6:26679.
- 3 Altekruse SF, McGlynn KA, Reichman ME: Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009;27:1485-1491.
- 4 Coleman MP, Forman D, Bryant H, Butler J, Rachet B, Maringe C, Nur U, Tracey E, Coory M, Hatcher J, McGahan CE, Turner D, Marrett L, Gjerstorff ML, Johannesen TB, Adolfsson J, Lambe M, Lawrence G, Meehan D, Morris EJ, Middleton R, Steward J, Richards MA: Cancer survival in Australia, Canada, Denmark, Norway, Sweden, and the UK, 1995-2007 (the International Cancer Benchmarking Partnership): An analysis of population-based cancer registry data. *Lancet* 2011;377:127-138.
- 5 Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z: Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009;10:25-34.
- 6 Yang LY, Chang RM, Lau WY, Ou DP, Wu W, Zeng ZJ: Mesohepatectomy for centrally located large hepatocellular carcinoma: Indications, techniques, and outcomes. *Surgery* 2014;156:1177-1187.
- 7 Oliveri RS, Wetterslev J, Gluud C: Hepatocellular carcinoma. *Lancet* 2012;380: 470-471.
- 8 Jing W, Luo P, Zhu M, Ai Q, Chai H, Tu J: Prognostic and diagnostic significance of SDPR-Cavin-2 in hepatocellular carcinoma. *Cell Physiol Biochem* 2016;39:950-960.
- 9 Singh S, Singh PP, Roberts LR, Sanchez W: Chemopreventive strategies in hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2014;11:45-54.
- 10 Jing W, Luo P, Zhu M, Ai Q, Chai H, Tu J: Prognostic and diagnostic significance of SDPR-Cavin-2 in hepatocellular carcinoma. *Cell Physiol Biochem* 2016;39:950-960.
- 11 Miura N, Wanaka A, Tohyama M, Tanaka K: MFH-1, a new member of the fork head domain family, is expressed in developing mesenchyme. *FEBS Lett* 1993;326:171-176.

- 12 Lin Y, McKinnon KE, Ha SW, Beck GJ: Inorganic phosphate induces cancer cell mediated angiogenesis dependent on forkhead box protein C2 (FOXC2) regulated osteopontin expression. *Mol Carcinog* 2015;54:926-934.
- 13 Watanabe A, Suzuki H, Yokobori T, Altan B, Kubo N, Araki K, Wada S, Mochida Y, Sasaki S, Kashiwabara K, Hosouchi Y, Kuwano H: Forkhead box protein C2 contributes to invasion and metastasis of extrahepatic cholangiocarcinoma, resulting in a poor prognosis. *Cancer Sci* 2013;104:1427-1432.
- 14 Cui YM, Jiang D, Zhang SH, Wu P, Ye YP, Chen CM, Tang N, Liang L, Li TT, Qi L, Wang SY, Jiao HL, Lin J, Ding YQ, Liao WT: FOXC2 promotes colorectal cancer proliferation through inhibition of FOXO3a and activation of MAPK and AKT signaling pathways. *Cancer Lett* 2014;353:87-94.
- 15 Li C, Ding H, Tian J, Wu L, Wang Y, Xing Y, Chen M: Forkhead box protein c2 promotes Epithelial-Mesenchymal transition, migration and invasion in Cisplatin-Resistant human ovarian cancer cell line (SKOV3/CDDP). *Cell Physiol Biochem* 2016;39:1098-1110.
- 16 Liu B, Jia Y, Cao Y, Wu S, Jiang H, Sun X, Ma J, Yin X, Mao A, Shang M: Overexpression of phosphoserine aminotransferase 1 (PSAT1) predicts poor prognosis and associates with tumor progression in human esophageal squamous cell carcinoma. *Cell Physiol Biochem* 2016;39:395-406.
- 17 Hollier BG, Tinnirello AA, Werden SJ, Evans KW, Taube JH, Sarkar TR, Sphyris N, Shariati M, Kumar SV, Battula VL, Herschkowitz JI, Guerra R, Chang JT, Miura N, Rosen JM, Mani SA: FOXC2 expression links epithelial-mesenchymal transition and stem cell properties in breast cancer. *Cancer Res* 2013;73:1981-1992.
- 18 Li Q, Wu J, Wei P, Xu Y, Zhuo C, Wang Y, Li D, Cai S: Overexpression of forkhead Box C2 promotes tumor metastasis and indicates poor prognosis in colon cancer via regulating epithelial-mesenchymal transition. *Am J Cancer Res* 2015;5:2022-2034.
- 19 Mani SA, Yang J, Brooks M, Schwaninger G, Zhou A, Miura N, Kutok JL, Hartwell K, Richardson AL, Weinberg RA: Mesenchyme Forkhead 1 (FOXC2) plays a key role in metastasis and is associated with aggressive basal-like breast cancers. *Proc Natl Acad Sci U S A* 2007;104:10069-10074.
- 20 Pietila M, Vijay GV, Soundararajan R, Yu X, Symmans WF, Sphyris N, Mani SA: FOXC2 regulates the G2/M transition of stem cell-rich breast cancer cells and sensitizes them to PLK1 inhibition. *Sci Rep* 2016;6:23070.
- 21 Reynolds AB: P120-catenin: Past and present. *Biochim Biophys Acta* 2007;1773:2-7.
- 22 Nishida N, Mimori K, Yokobori T, Sudo T, Tanaka F, Shibata K, Ishii H, Doki Y, Mori M: FOXC2 is a novel prognostic factor in human esophageal squamous cell carcinoma. *Ann Surg Oncol* 2011;18:535-542.
- 23 Gan L, Liu Z, Jin W, Zhou Z, Sun C: Foxc2 enhances proliferation and inhibits apoptosis through activating Akt/mTORC1 signaling pathway in mouse preadipocytes. *J Lipid Res* 2015;56:1471-1480.