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Loss of Secreted Frizzled-Related Protein-1 expression is associated with poor prognosis in intrahepatic cholangiocarcinoma

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Abstract

Aims: Secreted Frizzled-Related Protein-1 (SFRP1) is a well-known negative regulator of the wingless type (Wnt)-β-catenin pathway and its inactivation plays an important role in the development and progression of many cancers. In this study, we aimed to determine the clinical significance of SFRP1 expression in intrahepatic cholangiocarcinoma (IHCC) and to define the relationship to Wnt-β-catenin pathway.
Methods: Fifty IHCC patients who had liver resection were enrolled in this study. SFRP1 protein expression was examined by immunohistochemistry in tumor tissues. The patients were divided into two groups: SFRP1 positive (n = 30) and negative (n = 20). Clinicopathological characteristics were analyzed.
Results: SFRP1 significantly correlated with curability (Cur A, B vs. C, $p = 0.029$); and recurrent pattern (intrahepatic vs. extrahepatic, $p = 0.010$). The negative SFRP1 group had significantly poorer prognosis, and 5-year survival rates were 8.1% of the negative SFRP1 group and 44.6% of the positive SFRP1 group, respectively. Moreover, the disease-free survival rate in the negative SFRP1 group was significantly poorer ($p < 0.001$). Multivariate analysis revealed that loss of SFRP1served as an independent prognostic factor in IHCC for both overall (HR, 2.923; 95% CI, 1.30–6.56; $p = 0.009$) and disease-free (HR, 2.631; 95% CI, 1.31–5.27; $p = 0.006$) survival. In addition, SFRP1 expression negatively correlated to β-catenin expression ($p = 0.005$).
Conclusions: Those results suggested that the loss of SFRP1 could be a poor prognostic factor for IHCC, through the Wnt-β-catenin pathway.

Keywords: Intrahepatic cholangiocarcinoma; Prognostic factor; SFRP1; β-Catenin

Introduction

Intrahepatic cholangiocarcinoma (IHCC) is a distinct type of biliary tract cancer, which develops from the malignant conversion of intrahepatic cholangiocytes and possesses highly aggressive tumor biology. Despite being considered an uncommon malignancy, it was reported that the incidence of IHCC has been steadily increasing worldwide in the past decade and is currently defined as second only to hepatocellular carcinoma (HCC) as the most common hepatic malignancy, with a prevalence of 5–10% in all primary liver cancers.[1–4] Surgical resection is still considered to be only chance for cure and effective anti-cancer drug including molecular targeted agent has not been established. However, after curative operation has been completely performed, IHCC patients have a worse prognosis compared with other cancers.[5,6] Therefore, new insights into the biology of IHCC and identification of novel potential biomarkers are required for cancer management and treatment to improve the prognosis of patients with IHCC.

Abbreviations: IHCC, intrahepatic cholangiocarcinoma; HCC, hepatocellular carcinoma; Wnt, wingless-type; SFRP1, Secreted Frizzled-Related Protein-1; OS, overall survival; DFS, disease-free survival; IHC, immunohistochemistry; PBS, phosphate-buffered saline; DAB, 3,3′-diaminobenzidine-tetrachloride.

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The wingless type (Wnt) proteins are a family of lipid modified secreted auto-paracrine proteins, and interact with the extracellular cysteine-rich domain of 7 transmembrane receptors of the Frizzled Receptors, which leads to the expression of its target Wnt responsive genes.[7–9] During several Wnt related genes, β-catenin is one of critical parts of the canonical Wnt pathway.[7] When Wnt ligands binds to their receptor, it triggers a rise of the level of β-catenin.[10] Dephosphorylated β-catenin then aggregates in the cytoplasm followed by nuclear translocation and induces the expression of Wnt-responsive genes including several oncogenes, such as v-MYCyc avian myelocytomatosis viral oncogene homolog (C-Myc), JUN proto-oncogene (C-Jun), and Cyclin D1.[11–13] It has been recently reported that the atypical activation of the Wnt pathway is closely related to the tumorogenesis of various human cancers.[14–16]

A member of the negative regulators of the Wnt signaling pathway, Secreted Frizzled-Related Protein-1 (SFRP1) is located at chromosome 8p12−11.1 and is transcribed into a secreting glycoprotein that has two[1] cysteine-rich domains, called netrin domain and cysteine-rich domain (CRD), which are homologous to the frizzled receptors. Increasingly, evidence has revealed that the appropriate balance of Wnt signaling pathway and its antagonist Secreted Frizzled-Related Protein-1 (SFRP1) played an important role in various kinds of cancers.[17–20] Several studies reported the loss of SFRP1 in a variety of malignancies.[21–25] SFRP1was also recently demonstrated to be a new tumor suppressor that is inactivated by promoter methylation.

However, to the best our knowledge, we do not as yet known the expression pattern of SFRP1 in tumor tissue and its relationship to the prognosis of patients with IHCC. Therefore, we conducted this study to investigate the clinical significance of SFRP1 expression in IHCC and to define the relationship to Wnt-β-catenin pathway.

Patients and methods

Patients and specimens

A total of 50 patients with IHCC, who underwent surgical resection at Tokushima University Hospital between April 1994 and August 2013 were included in this study. The Ethical Committee of Tokushima University approved the study and all patients given written informed allowance. Patients' information was obtained from the medical records of the institute. Among all, 33 of the patients were men and 17 were women, with a mean age of 68.04 years. Curability and staging were defined according to the Classification of the Primary Liver Cancer Study Group of Japan.[26] In regards to the stages, factors were determined by tumor sizes; vascular invasion (absent or present); and tumor numbers (single or multiple). A stage was determined by Tumor-Node-Metastasis (T,N, and M) factors. The definition of curability was done as follows: curability A, no residual tumor for stage I and II patients; curability B, no residual tumor for stage III and IV patients; and finally, curability C, clear residual tumors. Among all of the 50 patients, 36 (72.0%) of them received curability A or B resections. Overall survival (OS) was defined as the interval between the dates of surgery and death. Disease-free survival (DFS) was defined as the interval between the dates of surgery and recurrence. If recurrence was not diagnosed, patients were censored on the date of death or the last follow-up. All patients were regularly followed up in the hospital out-patient clinic and checked prospectively for recurrence following a standard method. No one had received radiation therapy or chemotherapy before operation. The 3- and 5-years survival rates were 39.6% and 31.3%, respectively. Mean follow-up period was 38.7 months (range: 2.3−185.9 months).

Immunohistochemistry

The tissues were fixed in neutral buffered 10% formalin for 24 h and then embedded in paraffin. Then paraffin sections were used at 5 μm of intervals for. The sections were deparaffinized in xylene and rehydrated in a graded series of alcohol solution. Those sections were incubated in 3% H_2O_2 for 20 min to block endogenous peroxidase. Antigen retrieval of the sections was accomplished in a multifunctional microwave histoprocessor at 98 °C in ph 6.0 citrate buffer for 24 min and cooled at room temperature for 90 min. The sections were then incubated with rabbit polyclonal antibody against SFRP1 (ab4193, Abcam, Cambridge, UK; dilution at 1:200) and monoclonal antibody against β-catenin (sc-7963, Santa Cruz Biotechnology, Santa Cruz, CA; dilution at 1:100) for 60 min at room temperature. After washing 3 times in phosphate-buffered saline (PBS), the sections were incubated with a secondary horseradish peroxidase-tagged antibody labeled with anti-rabbit/mouse polymers (DAKO A/S, Glostup, Denmark) for 60 min at room temperature. The sections were then incubated with 3,3′-diaminobenzidine-tetrachloride (DAB) as chromogen following washing 3 times in PBS. Finally, all sections were counterstained with Mayer's hematoxylin solution and the sections were then dehydrated by a series of xylene and alcohol washes. Human breast cancer and human colon cancer were used as positive control for primary antibodies of SFRP1 and β-catenin, respectively, and PBS treated sections were used as negative control.

Assessment of immunohistochemistry

A pathologist who was blind to the clinical data visually scored stained slides. SFRP1 and β-catenin expressions were scored by staining intensity (0, negative; 1, weak, 2 moderate, and 3 strong) according to the previous reports.[21,27] SFRP1 scores of 2 or more were regarded as positive (Figure 1A), and also β-catenin scores of 2 or more

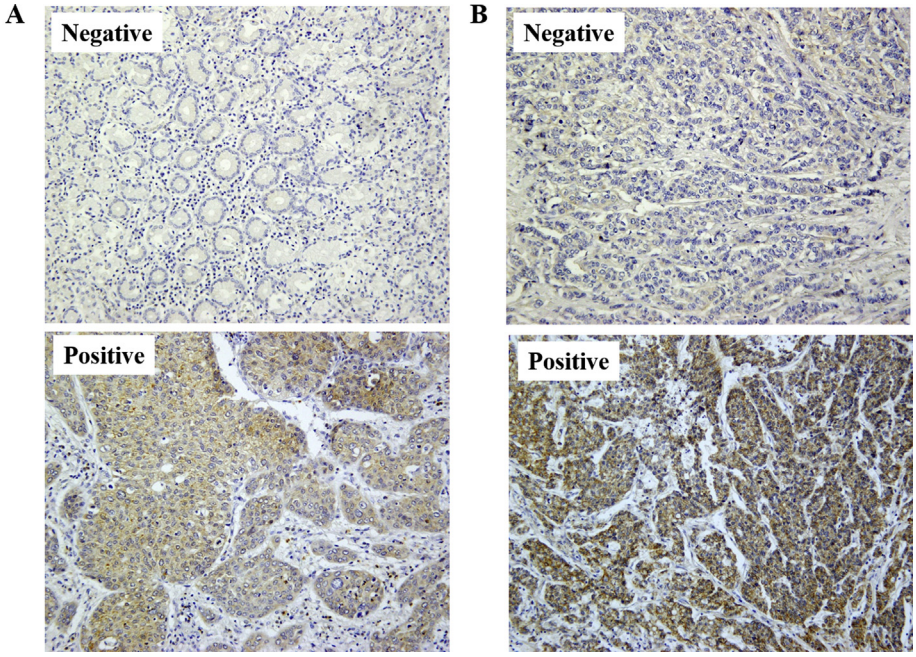

Figure 1. **Immunohistochemistry in IHCC tumor tissue**. (A) SFRP1, (B) β-catenin.

were regarded as positive (Figure 1B). The slides were evaluated in 5 different fields using Olympus BX43F photomicroscope at a magnification of ×200, and at least 1000 tumor cells were evaluated in each slide. SFRP1 expression was detected mainly in the cytoplasm, while β-catenin expression was detected in cytomembrane and nucleus. Consequently, according to that assessment of staining intensity, 20 (40.0%) of the 50 specimens were classified as negative for SFRP1. 30 (60.0%) were classified as positive. Regarding β-catenin, 27 (54.0%) of the 50 specimens were evaluated negative and 23 (46.0%) were positive.

Statistical analysis

We performed statistical analyses by using SPSS Version 21.0 statistical software (SPSS, Chicago, IL). The chi-square tests were used to define the relationship between SFRP1 versus β-catenin expressions and clinicopathological characteristics. Survival curves were generated by the Kaplan–Meier method and differences were compared by the log-rank test. Multivariate analysis was performed based on the Cox's proportional hazard model. For all statistical analyses, $p < 0.05$ was considered as significant.

Results

The relationship between SFRP1 expression and clinicopathological characteristics

In Table 1, clinicopathological variables according to the SFRP1 expression were shown. Negative SFRP1 was significantly correlated with non-curative resection ($p = 0.029$), and presence of extrahepatic metastasis ($p = 0.010$). However, there was no statistically significant correlation between SFRP1 expression and histological type; vascular invasion; lymph node metastasis; or the others.

Table 1
Clinicopathological characteristics according to SFRP 1 expression.

Factors		SFRP 1 expression		*p*-Value
		Negative (n = 20)	Positive (n = 30)	
Age	Mean ± SD (years)	68.8 ± 10.3	67.6 ± 9.4	0.273
Sex	Male/female	12/8	21/9	0.465
Hepatic viral infection	Negative/HBV/HCV/combined	14/3/3/0	21/6/2/1	0.644
Curability	A, B/C	11/9	25/5	0.029
Stage	I, II/III, IV	5/15	11/19	0.386
Maximum tumor size	<5 cm/≥5 cm	11/9	19/11	0.556
Macroscopic type	MF/MF + PI	8/12	16/14	0.355
Location	Hilar/peripheral	8/12	9/21	0.465
Differentiation	Well/others	11/9	22/8	0.180
Lymph node metastasis	Negative/positive	11/9	20/10	0.405
Portal invasion	Negative/positive	11/9	19/11	0.556
Venous invasion	Negative/positive	18/2	23/7	0.229
Intrahepatic metastasis	Negative/positive	17/3	25/5	0.875
Recurrence pattern	Intrahepatic/extrahepatic	8/10	15/4	0.010

SD; standard deviation, HBV; hepatitis B virus, HCV; hepatitis C virus, MF; mass-forming type, MF + PI; mass-forming and periductal infiltrative type.

Prognostic significance of SFRP1 expression

Negative SFRP1 expression was significantly correlated with significant poorer prognosis in patients with IHCC, and 5-year survival rate of patients with negative and positive SFRP1 expression were 8.1% and 44.6%, respectively (Figure 2A). Univariate analyses revealed that tumor size ($p = 0.031$), curability ($p < 0.001$), macroscopic type ($p = 0.035$), tumor location ($p = 0.006$), stages ($p < 0.001$), lymph node metastasis ($p = 0.002$), portal invasion ($p = 0.001$), intrahepatic metastasis ($p = 0.004$) and SFRP1 expression ($p = 0.002$) were significant prognostic factors for the overall survival (Table 2). Multivariate analysis using Cox's hazard proportional model showed that hilar tumor location ($p = 0.016$), advanced stages (stage III, IV) ($p = 0.032$) and negative SFRP1 expression ($p = 0.009$) were independent poor prognostic factors for the overall survival of patients with IHCC (Table 3).

Regarding disease-free survival, negative SFRP1 expression was significantly correlated with significant poorer prognosis, and 5-year survival rate of patients with negative and positive SFRP1 expression were 5.4% and 30.5%, respectively (Figure 2B). Univariate analysis revealed that curability ($p < 0.001$), macroscopic type ($p = 0.014$), advanced stages ($p < 0.001$), lymph node metastasis ($p = 0.001$), portal invasion ($p = 0.001$), intrahepatic metastasis ($p = 0.013$) and SFRP1 expression ($p < 0.001$) were significant prognostic factors (Table 4). Multivariate analysis showed that the negative SFRP1 expression ($p = 0.006$) was the only independent poor prognostic factor for disease-free survival in patients with IHCC (Table 5).

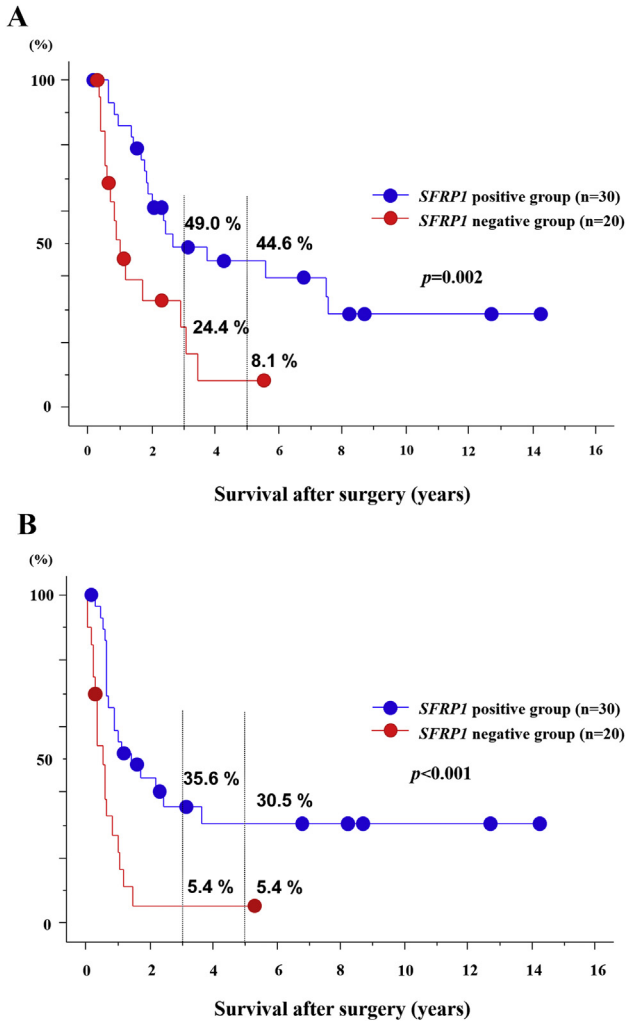

Figure 2. **Long term prognosis according to SFRP1 expression**. (A) Overall and (B) Disease-free survival curves with curative resection according to SFRP1 expression.

Table 2
Univariate analysis of risk factors for overall survival of patients.

Factors		3-Year survival (%)	*p*-Value
Age	<65 years/ ≥65 years	50.4% vs. 33.3%	0.437
Sex	Male/female	39.0% vs. 40.5%	0.796
Curability	A, B/C	52.1% vs. 00.0%	<0.001
Stage	I, II/III, IV	93.3% vs. 17.5%	<0.001
Maximum tumor size	<5 cm/≥5 cm	48.3% vs. 27.8%	0.031
Macroscopic type	MF/MF + PI	60.6% vs. 22.6%	0.035
Location	Hilar/peripheral	14.7% vs. 54.0%	0.006
Differentiation	Well/others	37.6% vs. 42.9%	0.971
Lymph node metastasis	Negative/positive	53.4% vs. 15.7%	0.002
Portal invasion	Negative/positive	56.2% vs. 16.0%	0.001
Venous invasion	Negative/positive	44.2% vs. 22.2%	0.235
Intrahepatic metastasis	Negative/positive	47.1% vs. 00.0%	0.004
SFRP1	Negative/positive	24.4% vs. 49.0%	0.002

MF; mass-forming type, MF + PI; mass-forming and periductal infiltrative type, SFRP1; Secreted Frizzled-Related Protein-1.

SFRP1 expression negatively correlated with β-catenin expression

To elucidate the possible pathway related with loss of SFRP1 toward the awful tumor biology in IHCC, we

Table 3
Multivariate analysis of risk factors for overall survival of patients.

Factors	Hazard ratio	95% CI	*p*-Value
Curability C	2.133	0.79–5.74	0.134
Stage III, IV	4.620	1.14–18.6	0.032
Maximum tumor size ≥5 cm	2.705	1.00–7.30	0.050
Macroscopic type MF+PI	1.806	0.58–5.57	0.303
Location hilar	3.095	1.23–7.78	0.016
Lymph node metastasis positive	1.602	0.55–4.64	0.38
Portal invasion positive	1.351	0.51–3.60	0.55
Intrahepatic metastasis positive	2.958	0.99–8.83	0.052
SFRP1 negative	2.923	1.30–6.56	0.009

MF; mass-forming type, MF + PI; mass-forming and periductal infiltrative type, SFRP1; Secreted Frizzled-Related Protein-1.

Table 4
Univariate analysis of risk factors for disease free survival of patients.

Factors		3-Year survival (%)	*p*-Value
Age	<65 years/ ≥65 years	33.6% vs. 18.2%	0.290
Sex	Male/female	20.9% vs. 30.4%	0.820
Curability	A, B/C	32.9% vs. 00.0%	<0.001
Stage	I, II/III, IV	63.3% vs. 06.1%	<0.001
Maximum tumor size	<5 cm/≥5 cm	27.6% vs. 17.0%	0.050
Macroscopic type	MF/MF + PI	39.9% vs. 9.0%	0.014
Location	Hilar/peripheral	11.8% vs. 30.8%	0.118
Differentiation	Well/others	19.8% vs. 31.7%	0.673
Lymph node metastasis	Negative/positive	38.5% vs. 00.0%	0.001
Portal invasion	Negative/positive	36.8% vs. 05.0%	0.001
Venous invasion	Negative/positive	26.7% vs. 11.1%	0.466
Intrahepatic metastasis	Negative/positive	27.6% vs. 00.0%	0.013
SFRP1	Negative/positive	5.4% vs. 35.6%	<0.001

MF; mass-forming type, MF + PI; mass-forming and periductal infiltrative type, SFRP1; Secreted Frizzled-Related Protein-1.

examined the relationship between SFRP1 and β-catenin in IHCC tumor tissues by chi-square tests. Consequently, among 30 SFRP1 positive patients, 21 patients (70%) indicated the negative β-catenin expression. Meanwhile only 6 of 20 SFRP1 negative patients (30%) presented the β-catenin expression negative, and these findings revealed that there was a significant negative correlation between SFRP1 and β-catenin expressions in IHCC ($p = 0.005$) (Figure 3).

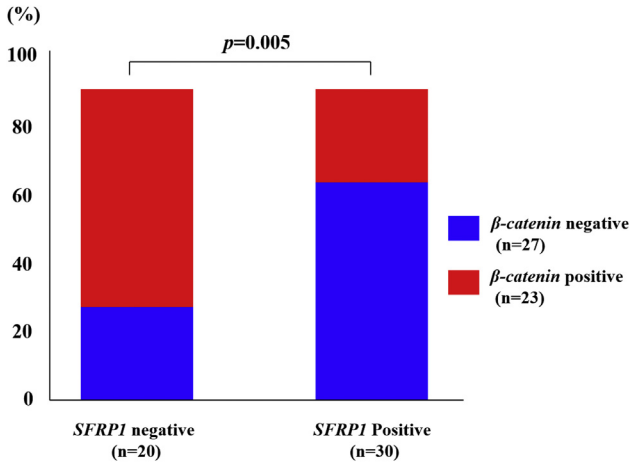

Figure 3. The relationship between SFRP1 and β-catenin.

Discussion

IHCC is a distinct and aggressive type of biliary tract cancer that arises from the cholangiocytes of small bile ducts and notch-mediated conversion of hepatocytes in the liver[28–30] and generally has a poor prognosis. Even though a lot of improvements have been made in the understanding of IHCC biology, the treatment choices are limited and their outcomes are still considered to be unsatisfactory.[5] Therefore, it is important to find novel predictive and prognostic biomarkers for IHCC. Some new therapeutic strategies including the improvement of surgical technique and perioperative management have been developing for the last two decades of patients who included in this study and it was possible to influence the patients' prognosis. However, during those periods, the innovative alteration for the treatment for this disease did not reach. Under such a circumstance, we previously reported that CD133, which was one of the most important cancer-initiating cell markers, was independently related to worse prognosis in patients with IHCC.[31] In this study, we demonstrated that low SFRP1 expression in IHCC tumor tissue was an independent poor prognostic factor in both overall and disease-free survival, and this molecular expression negatively correlated with β-catenin expression in immunohistochemical examination. Namely, those findings suggested that loss of SFRP1 could lead to aggressive tumor biology through the activation of Wnt-β-catenin pathway.

Table 5
Multivariate analysis of risk factors for disease free survival of patients.

Factors	Hazard ratio	95% CI	*p*-Value
Curability C	2.164	0.93–5.01	0.072
Stage III/IV	2.572	0.76–8.77	0.131
Macroscopic type MF+PI	1.764	0.75–4.16	0.195
Lymph node metastasis positive	1.037	0.42–2.57	0.937
Portal invasion positive	1.993	0.81–4.93	0.135
Intrahepatic metastasis positive	1.936	0.72–5.21	0.391
SFRP1 negative	2.631	1.31–5.27	0.006

MF; mass-forming type, MF + PI; mass-forming and periductal infiltrative type, SFRP1; Secreted Frizzled-Related Protein-1.

The Wnt pathway recognized to be a critical player in normal embryonic development or maintenance of adult tissues. In canonical activation of Wnt signaling pathway, its' major downstream molecule β-catenin is stabilized and translocated into the nucleus followed by the up-regulation of Wnt target genes such as C-Myc, C-Jun, and Cyclin D1.[10–13] Therefore, β-catenin stabilization and translocation are considered as major signs of Wnt signaling pathway activation in normal condition.[7] However, abnormal activation of the Wnt pathway was described in some kinds of cancers including HCC.[14–16,32] In our study, the expression of β-catenin was detected in the cytomembrane and nucleus in IHCC tumor tissue. Our results were consistent with some other reports in other cancers[27,33,34] and it might indicate that the aberrant activation of the Wnt signaling pathway and its major downstream molecule β-catenin could participate in a cruel clinical outcome of patients with IHCC.

SFRP1 is a secreting protein and a well-known antagonist of the Wnt signaling pathway by its inhibitory

functions of frizzled receptors, and has been reported to be a potential tumor suppressor in different kinds of cancers.[21–25,35–39] It has been widely reported that SFRP1 was silenced in various cancers,[40–42] suggesting that the aberrant loss of SFRP1 could be one of mechanism to activate the Wnt pathway in tumors. Furthermore, it was reported that epigenetically silenced SFRP1 might accelerate Wnt signaling pathway activation and thus stimulate the induction of epithelial–mesenchymal transition, which often led to the worsening of aggressiveness in several different HCC cell lines.[43] We detected that the SFRP1 expression had significant correlation with extrahepatic metastasis, and most importantly, SFRP1 negative patients had significantly worse both overall and disease-free survival rates in patients with IHCC. Moreover, multivariate analysis showed that SFRP1 might serve as an independent prognostic factor for overall and disease-free survival rates. Since SFRP1 is a well-known inhibitor of Wnt signaling pathway, we compared expression level of SFRP1 with expression level of β-catenin, which is major downstream molecule of Wnt signaling pathway and found that SFRP1 expression had negative correlation with β-catenin expression in IHCC, consistent with previous reports of human biliary tract carcinoma,[7] and prostate cancer.[27] Taken together, these results suggest that the loss of SFRP1 is a potential unfavorable prognostic biomarker for IHCC and this result is similar with some other studies done on different cancers.[7,27,38,44] Furthermore, this is a first report, which the loss of SFRP1 could lead to aggressive tumor biology through the activation of Wnt-β-catenin pathway.

Regarding the limitation of our study, we have checked only target proteins by immunohistochemistry and could not prove the mechanistic correlation between SFRP1 and Wnt-β-catenin pathway. Although we need to further identify, which kind of SFRP1 including its down-stream pathway is a responsible regulator for IHCC, any adjuvant chemotherapy after surgery should be provably introduced for the patients with loss of SFRP1 in tumor tissue at this stage.

In conclusion, our results suggested that the loss of SFRP1 expression in tumor tissue might be an independent prognostic factor that related to poorer overall and disease-free survival in patients with IHCC. SFRP1 and β-catenin are also inversely correlated with each other in our study. These findings recommend that loss of SFRP1 could be a potential prognostic biomarker for IHCC through the Wnt-β-catenin pathway.

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Conflict of interest

All authors have no financial or other interests in entities related to the subject of this article.

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References

1. Miura JT, Johnston FM, Tsai S, et al. Chemotherapy for surgically resected intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 2015;**22**:3716–23.
2. Xu YF, Ge FJ, Han B, et al. High-mobility group box 1 expression and lymph node metastasis in intrahepatic cholangiocarcinoma. *World J Gastroenterol* 2015;**21**:3256–65.
3. Zhang F, Li L, Yang X, et al. Expression and activation of EGFR and STAT3 during the multistage carcinogenesis of intrahepatic cholangiocarcinoma induced by 3′-methyl-4 dimethylaminoazobenzene in rats. *J Toxicol Pathol* 2015;**28**:79–87.
4. Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004;**24**:115–25.
5. Valle J, Wasan H, Palmer DH, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010;**362**:1273–81.
6. Enkhbold C, Saito Y, Morine Y, et al. Loss of FBXW7 expression is associated with poor prognosis in intrahepatic cholangiocarcinoma. *Hepatol Res* 2014;**44**:E346–52.
7. Kang P, Wan M, Huang P, et al. The Wnt antagonist sFRP1 as a favorable prognosticator in human biliary tract carcinoma. *PLoS One* 2014;**9**:e90308.
8. Pez F, Lopez A, Kim M, Wands JR, Caron de Fromentel C, Merle P. Wnt signaling and hepatocarcinogenesis: molecular targets for the development of innovative anticancer drugs. *J Hepatol* 2013;**59**:1107–17.
9. Stewart DJ, Chang DW, Ye Y, et al. Wnt signaling pathway pharmacogenetics in non-small cell lung cancer. *Pharmacogenomics J* 2014;**14**:509–22.
10. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. *Nat Rev Genet* 2004;**5**:691–701.
11. Behrens J, von Kries JP, Kuhl M, et al. Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* 1996;**382**:638–42.
12. Mann B, Gelos M, Siedow A, et al. Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. *Proc Natl Acad Sci U S A* 1999;**96**:1603–8.
13. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999;**398**:422–6.
14. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005;**434**:843–50.
15. King TD, Suto MJ, Li Y. The Wnt/beta-catenin signaling pathway: a potential therapeutic target in the treatment of triple negative breast cancer. *J Cell Biochem* 2012;**113**:13–8.
16. Schepers A, Clevers H. Wnt signaling, stem cells, and cancer of the gastrointestinal tract. *Cold Spring Harb Perspect Biol* 2012;**4**:a007989.
17. Caldwell GM, Jones C, Gensberg K, et al. The Wnt antagonist sFRP1 in colorectal tumorigenesis. *Cancer Res* 2004;**64**:883–8.
18. Zou H, Molina JR, Harrington JJ, et al. Aberrant methylation of secreted frizzled-related protein genes in esophageal adenocarcinoma and Barrett's esophagus. *Int J Cancer* 2005;**116**:584–91.
19. Veeck J, Niederacher D, An H, et al. Aberrant methylation of the Wnt antagonist SFRP1 in breast cancer is associated with unfavourable prognosis. *Oncogene* 2006;**25**:3479–88.
20. Liang J, Kang X, Halifu Y, et al. Secreted frizzled-related protein promotors are hypermethylated in cutaneous squamous carcinoma compared with normal epidermis. *BMC Cancer* 2015;**15**:641.

21. Dahl E, Wiesmann F, Woenckhaus M, et al. Frequent loss of SFRP1 expression in multiple human solid tumours: association with aberrant promoter methylation in renal cell carcinoma. *Oncogene* 2007;**26**:5680–91.
22. Lee AY, He B, You L, et al. Expression of the secreted frizzled-related protein gene family is downregulated in human mesothelioma. *Oncogene* 2004;**23**:6672–6.
23. Takada T, Yagi Y, Maekita T, et al. Methylation-associated silencing of the Wnt antagonist SFRP1 gene in human ovarian cancers. *Cancer Sci* 2004;**95**:741–4.
24. Vincan E, Barker N. The upstream components of the Wnt signalling pathway in the dynamic EMT and MET associated with colorectal cancer progression. *Clin Exp Metastasis* 2008;**25**:657–63.
25. Gauger KJ, Schneider SS. Tumour supressor secreted frizzled related protein 1 regulates p53-mediated apoptosis. *Cell Biol Int* 2014;**38**:124–30.
26. Japan LCSGo. *Classification of primary liver cancer.* 1st English edition. Tokyo: Kanehara; 1997.
27. Zheng L, Sun D, Fan W, Zhang Z, Li Q, Jiang T. Diagnostic value of SFRP1 as a favorable predictive and prognostic biomarker in patients with prostate cancer. *PLoS One* 2015;**10**:e0118276.
28. Sekiya S, Suzuki A. Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. *J Clin Invest* 2012;**122**:3914–8.
29. Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008;**48**:308–21.
30. Sia D, Tovar V, Moeini A, Llovet JM. Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. *Oncogene* 2013;**32**:4861–70.
31. Shimada M, Utsunomiya T, Morine Y, et al. CD133 expression is a potential prognostic indicator in intrahepatic cholangiocarcinoma. *J Gastroenterol* 2010;**45**:896–902.
32. Kaur P, Mani S, Cros MP, et al. Epigenetic silencing of sFRP1 activates the canonical Wnt pathway and contributes to increased cell growth and proliferation in hepatocellular carcinoma. *Tumour Biol* 2012;**33**:325–36.
33. Lin SY, Xia W, Wang JC, et al. Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. *Proc Natl Acad Sci U S A* 2000;**97**:4262–6.
34. Inagawa S, Itabashi M, Adachi S, et al. Expression and prognostic roles of beta-catenin in hepatocellular carcinoma: correlation with tumor progression and postoperative survival. *Clin Cancer Res* 2002;**8**:450–6.
35. Finch PW, He X, Kelley MJ, et al. Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. *Proc Natl Acad Sci U S A* 1997;**94**:6770–5.
36. Bafico A, Gazit A, Pramila T, Finch PW, Yaniv A, Aaronson SA. Interaction of frizzled related protein (FRP) with Wnt ligands and the frizzled receptor suggests alternative mechanisms for FRP inhibition of Wnt signaling. *J Biol Chem* 1999;**274**:16180–7.
37. Uren A, Reichsman F, Anest V, et al. Secreted frizzled-related protein-1 binds directly to Wingless and is a biphasic modulator of Wnt signaling. *J Biol Chem* 2000;**275**:4374–82.
38. Huang J, Zhang YL, Teng XM, et al. Down-regulation of SFRP1 as a putative tumor suppressor gene can contribute to human hepatocellular carcinoma. *BMC Cancer* 2007;**7**:126.
39. Gumz ML, Zou H, Kreinest PA, et al. Secreted frizzled-related protein 1 loss contributes to tumor phenotype of clear cell renal cell carcinoma. *Clin Cancer Res* 2007;**13**:4740–9.
40. Chen YZ, Liu D, Zhao YX, Wang HT, Gao Y, Chen Y. Aberrant promoter methylation of the SFRP1 gene may contribute to colorectal carcinogenesis: a meta-analysis. *Tumour Biol* 2014;**35**:9201–10.
41. Ren XY, Zhou GQ, Jiang W, et al. Low SFRP1 expression correlates with poor prognosis and promotes cell invasion by activating the Wnt/beta-catenin signaling pathway in NPC. *Cancer Prev Res (Phila)* 2015;**8**:968–77.
42. Rogler A, Kendziorra E, Giedl J, et al. Functional analyses and prognostic significance of SFRP1 expression in bladder cancer. *J Cancer Res Clin Oncol* 2015;**141**:1779–90.
43. Quan H, Zhou F, Nie D, et al. Hepatitis C virus core protein epigenetically silences SFRP1 and enhances HCC aggressiveness by inducing epithelial-mesenchymal transition. *Oncogene* 2014;**33**:2826–35.
44. Majchrzak-Celinska A, Slocinska M, Barciszewska AM, Nowak S, Baer-Dubowska W. Wnt pathway antagonists, SFRP1, SFRP2, SOX17, and PPP2R2B, are methylated in gliomas and SFRP1 methylation predicts shorter survival. *J Appl Genet* 2016;**57**:189–97.