Received: 5 November 2019 | Revised: 14 November 2019 | Accepted: 18 November 2019

DOI: 10.1002/jcla.23138

RESEARCH ARTICLE

WILEY

Circular RNA SMARCA5 correlates with favorable clinical tumor features and prognosis, and increases chemotherapy sensitivity in intrahepatic cholangiocarcinoma

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Abstract

Objective: This present study aimed to investigate the correlation of circular RNA SMARCA5 (circ-SMARCA5) with clinicopathological features and overall survival (OS), and the effect of circ-SMARCA5 on cell proliferation and chemotherapy sensitivity to cisplatin/gemcitabine in intrahepatic cholangiocarcinoma (ICC).

Methods: Totally 92 primary ICC patients who underwent resection were recruited, and their tumor tissues and adjacent tissues were collected for circ-SMARCA5 detection. The effect of circ-SMARCA5 on cell proliferation and chemotherapy sensitivity was detected after circ-SMARCA5 overexpression plasmid transfection into TFK-1 and HuH-28 ICC cells.

Results: Circ-SMARCA5 expression was reduced in ICC tumor tissues compared to adjacent tissues. Tumor circ-SMARCA5 high expression was negatively associated with Eastern Cooperative Oncology Group performance score, T stage, N stage, TNM stage, and abnormal CA199 status. Furthermore, OS was increased in patients with tumor circ-SMARCA5 high expression compared with those with low expression, and further multivariate Cox's regression demonstrated that tumor circ-SMARCA5 high expression was an independent predictive factor for longer OS. In TFK-1 and HuH-28 ICC cells, circ-SMARCA5 upregulation decreased cell proliferation, reduced relative cell viability in cisplatin-treated as well as gemcitabine-treated cells, and also decreased inhibitory concentration by 50% value (IC_{50}) of cisplatin and gemcitabine.

Conclusion: The correlation of circ-SMARCA5 with favorable clinical tumor features, survival profile, and its promoting effect on chemotherapy sensitivity implies its potential as a valuable biomarker in monitoring disease progression and prognosis of ICC.

KEYWORDS

cell proliferation, chemotherapy sensitivity, circular RNA SMARCA5, intrahepatic cholangiocarcinoma, prognosis

1 | INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC), as the second most common primary liver cancer, is a hepatobiliary malignancy located proximally to the second degree bile ducts, and its incidence is increasing in the last 20 years.[1,2] Surgical resection is considered to be the only potentially curative treatment with 5-year survival rates ranging from 30% to 40% and remains as the cornerstone of therapy for patients with ICC.[3] Besides, other treatments consist of neoadjuvant/adjuvant therapy, intensive cytotoxic therapy, hepatic arterial infusion therapy, embolization therapy, etc, which help improve clinical outcomes in the patients with ICC.[4-7] However, there are still a large number of patients with ICC suffering from poor long-term survival due to a high risk of recurrence, node metastasis, vascular invasion, and increasing chemotherapy resistance.[3,5] Therefore, research focusing on the discovery of novel biomarkers is essential, which can be used in the clinical application and improve the long-term therapeutic efficacy.

Accumulating evidence indicates that circular RNAs (circRNAs) can be generated from intronic, intergenic, coding, and 5′- or 3′-untranslational regions, and their molecular functions consist of serving as microRNA sponge, regulating transcription and splicing, adapting protein-protein interaction, etc, which possesses several biology functions of circRNA in cellular differentiation, physiological homeostasis, and even tumorigenesis.[8,9] Circular RNA SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 5 (circ-SMARCA5) is a circRNA derived from exons 15 and 16 of SMARCA5 gene, and SMARCA5 gene has effect on regulating activities of helicase and ATPase, remodeling chromatin, and facilitating the transcription of class II genes.[10] Previous published papers indicate that circ-SMARCA5 is downregulated and exerts anti-tumor effects in various cancers, such as HCC, non–small-cell lung cancer, gastric cancer, and cervical cancer.[11-14] For example, in HCC, circ-SMARCA5 is downregulated in HCC tissues, and its downregulation is associated with aggressive tumor features as well as unfavorable survival profiles in HCC patients.[13,15] And in vivo experiments reveal that circ-SMARCA5 inhibits HCC cell proliferation and migration.[15] Considering that ICC and HCC share some similarities in molecular profiles, dominant risk factors, and clinical manifestations, therefore, we speculated that circ-SMARCA5 might be involved in the ICC development and progression.[16,17] In the current study, we aimed to investigate the correlation of circ-SMARCA5 expression with clinical characteristics and survival profiles in patients with ICC and further conducted the cellular experiments to discover the role of circ-SMARCA5 in regulating cell proliferation and chemosensitivity of ICC.

2 | MATERIALS AND METHODS

2.1 | Patients

This prospective study consecutively enrolled 92 primary ICC patients who underwent resection in our hospital between July 2014 and June 2016. The inclusion criteria were (a) newly diagnosed as primary ICC by imageology and histopathology; (b) 18 years ≤ age ≤ 80 years; (c) clinical condition was suitable for surgery and about to receive resection; and (d) without other malignancies. The excluded criteria were (a) HCC or mixed HCC-ICC; (b) received neoadjuvant therapy before enrollment; (c) with distant metastasis; (d) history of human immunodeficiency virus infection; and (e) pregnant or lactating women. This study was approved by Ethics Committee of our hospital and was conducted according to the principles expressed in the Declaration of Helsinki. All patients or their guardians provided the written informed consents.

2.2 | Data and sample collection

The clinical characteristics of patients were recorded after enrollment, which included age, gender, smoke, drink, hepatitis B virus (HBV) infection, Eastern Cooperative Oncology Group (ECOG) performance score, pathological differentiation, T stage, N stage, TNM stage, carcinoembryonic antigen (CEA) level, and carbohydrate antigen 199 (CA199) level. Tumor tissue and paired adjacent tissue resected from surgery were stored in liquid nitrogen, immediately. And the tumor tissue and adjacent tissue were divided into two parts, one was used for pathological diagnosis, and another was used for circ-SMARCA5 detection in this study.

2.3 | Reverse transcription quantitative polymerase chain reaction

The relative expression of circ-SMARCA5 in tumor tissue and adjacent tissue was detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was firstly extracted from tumor tissues and adjacent tissues using TRIzol Reagent (Invitrogen), and linear RNA was removed using RNase R (Epicentre). Following that, RNA was reversely transcribed to cDNA using iScript™ cDNA Synthesis Kit (Bio-Rad). Finally, qPCR was conducted using THUNDERBIRD® SYBR® qPCR Mix (Toyobo). All the procedures were carried out according to the protocols of manufacturers. GAPDH was identified and evaluated as a suitable reference gene for circRNA expression normalization.[18] Then, circ-SMARCA5 expression was calculated using $2^{-\Delta\Delta Ct}$ with GAPDH as internal reference. The primer sequences used for RT-qPCR were listed as followed: Circ-SMARCA5 forward: ACAATGGATACAGAGTCAAG, reverse: CTTCATCAGTGATCTCACT; GAPDH forward: TGACCACAGTCCATGCCATCAC, reverse: GCCTGCTTCACCACCTTCTTGA.

2.4 | Grouping

According to the relative expression of circ-SMARCA5 in tumor tissue, all patients were classified as circ-SMARCA5 high expression

group (50%-100% quantile of circ-SMARCA5 relative expression in tumor tissue, n = 46) and circ-SMARCA5 low expression group (0%-50% quantile of circ-SMARCA5 relative expression in tumor tissue, n = 46). In addition, the circ-SMARCA5 low expression group was further divided into circ-SMARCA5 low- expression group (25%-50% quantile of circ-SMARCA5 relative expression in tumor tissue, n = 23), circ-SMARCA5 low-- expression group (10%-25% quantile of circ-SMARCA5 relative expression in tumor tissue, n = 14), and circ-SMARCA5 low--- expression group (0%-10% quantile of circ-SMARCA5 relative expression in tumor tissue, n = 9).

2.5 | Treatment and follow-up

After resection, all patients received gemcitabine/cisplatin combination adjuvant treatment based on the status of margins, according to NCCN Guidelines for Intrahepatic Cholangiocarcinoma.[20] Continuous follow-up was carried out for the patients until 2019/06/30. The median duration of follow-up was 26.5 months ranging from 2.0 to 60.0 months. Overall survival (OS) was defined as the duration from enrollment to death, and for the patients not known to have died in the last follow-up date, they were censored on the date of last known to be alive. Because most patients in the current study were non-local patients, the precise disease status of them was unable to acquire timely; consequently, the disease-free survival (DFS) was not evaluated.

2.6 | Cell culture

Human ICC cell line TFK-1 was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen, while another human ICC cell line HuH-28 was purchased from Japanese Cancer Research Resources Bank—Cell Bank. TFK-1 cells were cultured in 90% Roswell Park Memorial Institute (RPMI) 1640 Medium (Gibco) and 10% fetal bovine serum (FBS; Gibco) under 95% air 5% CO_2 at 37°C, while HuH-28 cells were cultured in 80% RPMI 1640 Medium (Gibco, USA) and 20% FBS (Gibco) under 95% air 5% CO_2 at 37°C.

2.7 | Transfection

The pCD5-ciR vector (Geneseed Biotech Co., Ltd) was used to structure circ-SMARCA5 overexpression (OE) plasmid and control OE plasmid. After construction, the OE plasmids were transfected into HuH-28 cells and TFK-1 cells using Lipofectamine 2000 (Thermo); then, the cells transfected with circ-SMARCA5 OE plasmids were marked as OE-Circ group; and correspondingly, the cells transfected with control OE plasmids were termed as OE-Control group. After transfection, cell proliferation in both groups was measured at 0, 24, 48, and 72 hours using Cell Counting Kit-8 (Sigma), and the procedure was in accordance with manufacturer's manual.

2.8 | Chemosensitivity

At 24 hours after transfection, the OE-Circ cells and OE-Control cells were plated in 96-multi-well plates with a concentration of 5000 cells/well. Then, the cells were treated with several concentrations of Cisplatin (Sigma) and Gemcitabine (Sigma) for 72 hours, respectively. After 72 hours treatment, the cell viability of the OE-Circ cells and OE-Control cells at different drug concentrations was determined by Cell Counting Kit-8 (Sigma), and the procedure was in accordance with manufacturer's manual. The cells treated with 0 μmol/L Cisplatin or 0 μmol/L Gemcitabine in each group were used as the reference in calculation of relative cell viability. Further, the drug concentration required to inhibit growth by 50% (IC_{50}) was calculated for all treated cells with the use of probit regression analysis.

2.9 | Statistical analysis

Continuous variables were displayed as mean ± standard deviation (SD) or median and interquartile range (IQR), and the categorical variables were expressed as count and percentage. Comparisons of unpaired variable between two groups were determined by Student's *t* test or chi-square test. Comparisons of paired variable between two groups were determined by Wilcoxon signed rank test. OS was illustrated using Kaplan-Meier curve, and the difference of OS between/among groups was determined by log-rank test. Factors predicting OS were analyzed by univariate Cox's proportional hazard regression model and forward stepwise multivariate Cox's regression model. All statistical analyses were performed using SPSS 22.0 software (IBM), and figures were made using GraphPad Prism 7.00 software (GraphPad Software). All tests were two-sided; *P* value < .05 was considered as significant.

3 | RESULTS

3.1 | Clinical characteristics of ICC patients

In total patients with ICC, the mean age was 59.2 ± 7.4 years, and there were 27 (29.3%) females and 65 (70.7%) males (Table 1). Regarding ECOG performance score, there were 57 (62.0%) patients with 0, and 35 (38.0%) patients with 1/2. As for pathological differentiation, there were 55 (59.8%) patients with well/moderate pathological differentiation, and 37 (40.2%) patients with poor pathological differentiation. The number of patients with I/II and III/IV TNM stage was 54 (58.7%) and 38 (41.3%), respectively. More detailed clinical characteristics were shown in Table 1.

TABLE 1 Clinical characteristics

Items	Total patients (N = 92)	Circ-SMARCA5		*P* value
		Low (n = 46)	High (n = 46)	
Age (y), mean ± SD	59.2 ± 7.4	58.5 ± 8.0	59.9 ± 6.8	.372
Gender, No. (%)				
Female	27 (29.3)	13 (28.3)	14 (30.4)	.819
Male	65 (70.7)	33 (71.7)	32 (69.6)	
Smoke, No. (%)	32 (34.8)	18 (39.1)	14 (30.4)	.381
Drink, No. (%)	41 (44.6)	18 (39.1)	23 (50.0)	.294
HBV infection, No. (%)	36 (39.1)	16 (34.8)	20 (43.5)	.393
ECOG performance score, No. (%)				
0	57 (62.0)	20 (43.5)	37 (80.4)	<.001
1/2	35 (38.0)	26 (56.5)	9 (19.6)	
Pathological differentiation, No. (%)				
Well and moderate	55 (59.8)	26 (56.5)	29 (63.0)	.524
Poor	37 (40.2)	20 (43.5)	17 (37.0)	
T stage, No. (%)				
T1/T2	74 (80.4)	33 (71.7)	41 (89.1)	.036
T3/T4	18 (19.6)	13 (28.3)	5 (10.9)	
N stage, No. (%)				
N0	56 (60.9)	17 (37.0)	39 (84.8)	<.001
N1	36 (39.1)	29 (63.0)	7 (15.2)	
TNM stage, No. (%)				
I/II	54 (58.7)	17 (37.0)	37 (80.4)	<.001
III/IV	38 (41.3)	29 (63.0)	9 (19.6)	
CEA abnormal,[a] No. (%)	37 (40.2)	20 (43.5)	17 (37.0)	.524
CA199 abnormal,[b] No. (%)	55 (59.8)	33 (71.7)	22 (47.8)	.019

Note: Comparison was determined by Student's *t* test or chi-square test.

Abbreviations: CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; HPV, hepatitis B virus; ICC, intrahepatic cholangiocarcinoma; SD, standard deviation.

[a]Abnormal, CEA > 5 ng/mL; normal, CEA ≤ 5 ng/mL.

[b]Abnormal, CA199 > 37 U/mL; normal, CA199 ≤ 37 U/mL.

3.2 | Comparison of Circ-SMAECA5 in ICC tumor tissues and pair adjacent tissues

Circ-SMARCA5 relative expression was reduced in ICC tumor tissues (0.311 [0.179-0.602]) compared with adjacent tissues (0.987 [0.709-1.297]; *P* < .001; Figure 1).

3.3 | Correlation of circ-SMAECA5 expression with clinical features in ICC patients

All patients with ICC were divided into circ-SMARCA5 high expression group (n = 46) and circ-SMARCA5 low expression group (n = 46) according to 50% quantile of circ-SMARCA5 relative expression in tumor tissue. Circ-SMARCA5 expression was negatively associated with ECOG performance score (*P* < .001), T stage (*P* < .036), N stage (*P* < .001), TNM stage (*P* < .001), and abnormal CA199 status (*P* = .019); however, there was no association of circ-SMARCA5 expression with age (*P* = .372), gender (*P* = .819), smoke (*P* = .381), drink (*P* = .294), HBV infection (*P* = .393), pathological differentiation (*P* = .524), or CEA (*P* = .524) in patients with ICC (Table 1).

3.4 | Correlation of circ-SMARCA5 expression with OS in ICC patients

OS was increased in patients with circ-SMARCA5 high expression compared with those with circ-SMARCA5 low expression (*P* = .007; Figure 2A). Furthermore, circ-SMARCA5 low expression group was divided into circ-SMARCA5 low- expression group (25%-50% quantile

of circ-SMARCA5 relative expression in tumor tissue), circ-SMARCA5 low-- expression group (10%-25% quantile of circ-SMARCA5 relative expression in tumor tissue), and circ-SMARCA5 low--- expression group (0%-10% quantile of circ-SMARCA5 relative expression in tumor tissue). OS was the highest in patients with circ-SMARCA5 high expression, followed by patients with circ-SMARCA5 low- expression and patients with circ-SMARCA5 low-- expression, and then patients with circ-SMARCA5 low--- expression ($P < .001$; Figure 2B).

3.5 | Factors affecting OS in ICC patients

Univariate Cox's regression analysis exhibited that circ-SMARCA5 high expression (HR = 0.535, $P = .008$) was associated with longer OS, while higher ECOG performance score (HR = 2.060, $P = .003$), poor pathological differentiation (HR = 2.896, $P < .001$), and advanced TNM stage (HR = 2.371, $P < .001$) were correlated with reduced OS in patients with ICC (Table 2). And forward stepwise multivariate Cox's regression analysis presented that circ-SMARCA5 high expression was an independent predictive factor for increased OS (HR = 0.518, $P = .006$), and poor pathological differentiation was an independent predictive factor for decreased OS (HR = 2.979, $P < .001$) in patients with ICC.

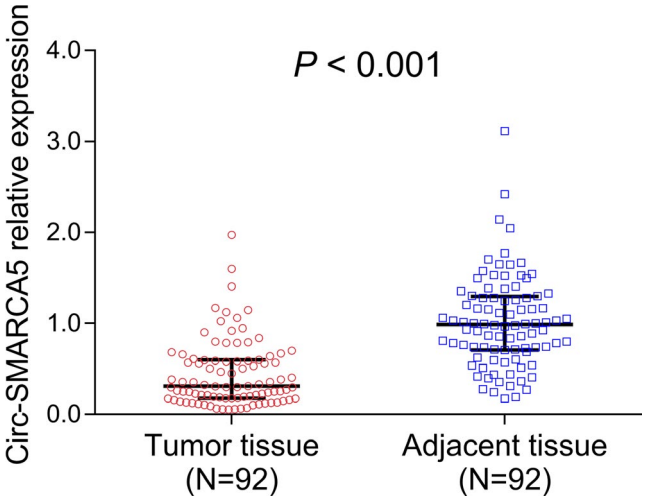

FIGURE 1 Circ-SMARCA5 expression was reduced in ICC tumor tissue compared with adjacent tissue. ICC, intrahepatic cholangiocarcinoma; Circ-SMARCA5, circular RNA SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 5

3.6 | Effect of circ-SMARCA5 upregulation on cell proliferation in ICC cells

We conducted the in vitro experiments to explore the effect of circ-SMARCA5 upregulation on cell proliferation in two ICC cell lines. In HuH-28 ICC cells, cell proliferation was reduced in OE-Circ group compared with OE-Control group at 48 hours ($P < .05$) and 72 hours ($P < .01$) after transfection (Figure 3A). As for in TFK-1 cells, cell proliferation was also decreased in OE-Circ group compared with OE-Control group at 48 hours ($P < .05$) and 72 hours ($P < .05$) after transfection (Figure 3B).

3.7 | Effect of circ-SMARCA5 upregulation on chemotherapy sensitivity to cisplatin/gemcitabine in ICC cells

Further experiments were performed to investigate the effect of circ-SMARCA5 upregulation on chemotherapy sensitivity to cisplatin/gemcitabine in two ICC cell lines. In HuH-28 cells, relative cell viability was decreased in OE-Circ group compared with OE-Control group in 4 μmol/L ($P < .05$), 8 μmol/L ($P < .05$), and 16 μmol/L ($P < .05$) cisplatin-treated cells (Figure 4A); relative cell viability was also reduced in OE-Circ group compared with OE-Control group in 4 μmol/L ($P < .01$), 8 μmol/L ($P < .01$), and 16 μmol/L ($P < .05$) gemcitabine-treated cells (Figure 4B). Furthermore, IC_{50} value of cisplatin ($P < .01$; Figure 4C)

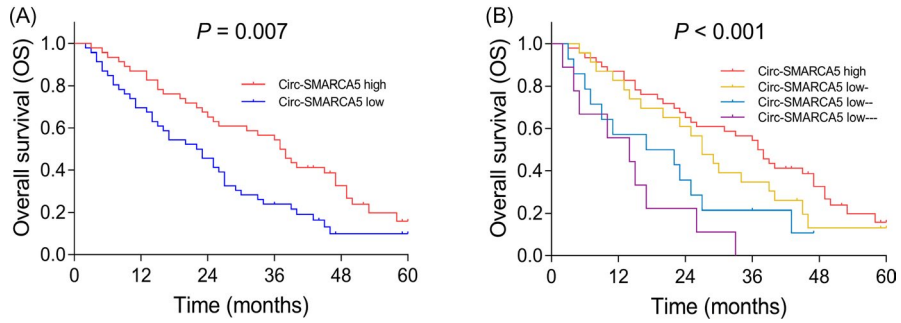

FIGURE 2 Circ-SMARCA5 positively associated with OS in patients with ICC. Comparison of OS between ICC patients with circ-SMARCA5 high expression and those with circ-SMARCA5 low expression (A). Comparison of OS among ICC patients with circ-SMARCA5 high expression, those with low- expression, those with low-- expression, and those with low---expression (B). OS, overall survival; ICC, intrahepatic cholangiocarcinoma; circ-SMARCA5, circular RNA SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 5; circ-SMARCA5 low- expression, 25%-50% quantile of circ-SMARCA5 relative expression in tumor tissue; circ-SMARCA5 low-- expression, 10%-25% quantile of circ-SMARCA5 relative expression in tumor tissue; and circ-SMARCA5 low--- expression, 0%-10% quantile of circ-SMARCA5 relative expression in tumor tissue

TABLE 2 Analysis of factors predicting OS

Items	Cox's proportional hazard regression model			
	P value	HR	95% CI	
			Lower	Higher
Univariate Cox's regression				
Circ-SMARCA5				
Low expression	Reference	—	—	—
High expression	.008	0.535	0.336	0.850
Age (y)				
≤60	Reference	—	—	—
>60	.713	1.089	0.691	1.717
Gender				
Female	Reference	—	—	—
Male	.689	1.113	0.659	1.878
Smoke				
No	Reference	—	—	—
Yes	.206	1.348	0.848	2.143
Drink				
No	Reference	—	—	—
Yes	.859	1.042	0.660	1.646
HBV infection				
No	Reference	—	—	—
Yes	.902	1.030	0.643	1.651
ECOG performance score				
0	Reference	—	—	—
1/2	.003	2.060	1.286	3.300
Pathological differentiation				
Well/moderate	Reference	—	—	—
Poor	<.001	2.896	1.787	4.692
TNM stage				
I/II	Reference	—	—	—
III/IV	<.001	2.371	1.484	3.787
CEA[a]				
Normal	Reference	—	—	—
Abnormal	.143	1.414	0.889	2.247
CA199[b]				
Normal	Reference	—	—	—
Abnormal	.130	1.438	0.899	2.303
Forward stepwise multivariate Cox's regression				
Circ-SMARCA5				
Low expression	Reference	—	—	—
High expression	.006	0.518	0.324	0.826
Pathological differentiation (poor)				
Well/moderate	Reference	—	—	—
Poor	<.001	2.979	1.828	4.855

Note: Factors predicting OS were analyzed by univariate Cox's proportional hazard regression model and forward stepwise multivariate Cox's regression model.

Abbreviations: CA199, carbohydrate antigen 199; CEA, carcinoembryonic antigen; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HPV, hepatitis B virus; HR, hazard ratio; OS, overall survival.

[a]CEA > 5 ng/mL; normal, CEA ≤ 5 ng/mL.

[b]Abnormal, CA199 > 37 U/mL; normal, CA199 ≤ 37 U/mL.

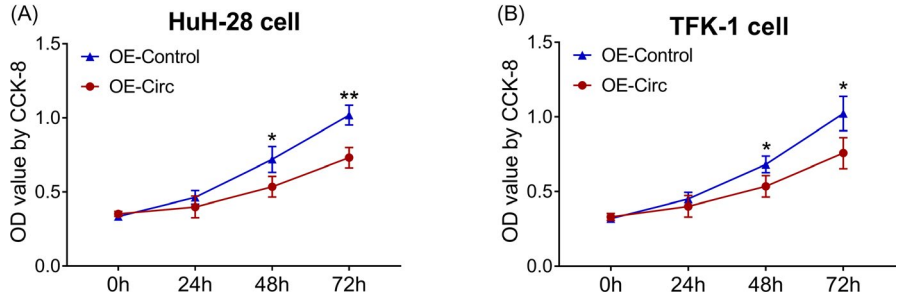

FIGURE 3 Circ-SMARCA5 inhibited proliferation in ICC cells after transfection. Comparison of OD value by CCK-8 between OE-Control group and OE-Circ in HuH-28 cells (A) and TFK-1 cells (B). ICC, intrahepatic cholangiocarcinoma; circ-SMARCA5, circular RNA SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 5; OE-Circ group, the cells transfected with circ-SMARCA5 overexpression plasmids; OE-Control group, the cells transfected with control overexpression plasmids; OD, optical density, CCK-8, Cell Counting Kit-8

and gemcitabine ($P < .001$; Figure 4D) was both decreased in OE-Circ group compared with OE-Control group. Similarly, in TFK-1 cells, relative cell viability was reduced in OE-Circ group compared with OE-Control group in 16 μmol/L ($P < .05$), 32 μmol/L ($P < .05$), and 64 μmol/L ($P < .05$) cisplatin-treated cells (Figure 4E); relative cell viability was also decreased in OE-Circ group compared with OE-Control group in 8 μmol/L ($P < .05$) and 16 μmol/L ($P < .05$) gemcitabine-treated cells (Figure 4F). IC_{50} value of cisplatin ($P < .01$; Figure 4G) and gemcitabine ($P < .01$; Figure 4H) was both decreased in OE-Circ compared with OE-Control. These suggested that circ-SMARCA5 upregulation promoted chemotherapy sensitivity to cisplatin/gemcitabine in ICC cells.

4 | DISCUSSION

In the current study, we found that (a) circ-SMARCA5 was downregulated in ICC tumor tissues compared with adjacent tissues; (b) circ-SMARCA5 was negatively associated with ECOG performance score, TNM stage, and abnormal CA199 status in patients with ICC. (c) circ-SMARCA5 was an independent predictive factor for increased OS in patients with ICC. (d) circ-SMARCA5 upregulation inhibited cell proliferation and increased cell chemotherapy sensitivity to cisplatin/gemcitabine in ICC.

CircRNAs have been identified to be expressed stably in various cells, and they are reported to be involved in various physiological development, including serving as microRNA sponges, interacting with proteins, regulating transcription and splicing, and participating in translation.[8,21,22] Until now, there are only preliminary investigations focusing on the role of circRNAs in cancers.[22] Existing evidence demonstrates that circRNA expression is abnormal expressed in tumor tissues compared with adjacent tissues, and associated with clinical outcomes in patients with tumors.[23-28] For example, CircRNA PTPRM expression is significantly increased in HCC tissues compared with paired adjacent tissues, and its overexpression is correlated with HCC recurrence and metastasis in HCC patients.[23] Another study exhibits that high level of circRNA IGF1R is correlated with tumor size in HCC patients.[25] Regarding circ-SMARCA5, as one of circRNAs, several publications reveal its participation in the tumorigenesis of various tumors, and circ-SMARCA5 high expression is reported to be correlated with favorable clinicopathological features and prognosis in patients with cancers.[11,12,14,29] For example, circ-SMARCS5 is greatly downregulated in gastric cancer tissues compared with adjacent noncancerous tissues and correlates with well differentiation, negative lymph node metastasis, no vascular invasion, and favorable prognosis in patients with gastric cancer.[14] In addition, circ-SMARCA5 is found to be downregulated in glioblastoma multiforme biopsies compared to normal brain tissues, and there exists a negative correlation between circ-SMARCA5 expression and histological grade in patients with glioma.[29] Furthermore, circ-SMARCA5 expression is decreased in HCC tissues compared with adjacent tissues, and its downregulation is correlated with advanced tumor features; furthermore, circ-SMARCA5 serves as an independent predictive factor for longer recurrence-free survival and OS in patients with HCC after hepatectomy.[13] However, the research on the role of circ-SMARCA5 in ICC has not conducted yet. Considering the similarities of cellular origin, genomic mutations, and risk factors between ICC and HCC, we speculated that circ-SMARCA5 might also be aberrantly expressed in ICC tissues and correlated with tumor features in patients with ICC.[16,17] We found that circ-SMARCA5 was downregulated in ICC tumor tissues compared with adjacent tissues and was negatively associated with ECOG performance score, TNM stage, and abnormal CA199 status in patients with ICC. The possible reasons might include that Circ-SMARCA5 might inhibit cell proliferation, migration, and invasion via acting as the miRNA sponge (such as miR-17-3p and miR-181b-5p, which served as targets of circ-SMARCA5 in HCC) in ICC, which contributed to the tumor-inhibitory effect on ICC progression; therefore, circ-SMARCA5 is correlated with favorable clinicopathological features in patients with ICC; and however, this speculation needed to be further verified by cellular experiments in ICC.[11] In addition, as for the correlation of circ-SMARCA5 with survival profile in patients with ICC, we observed that circ-SMARCA5 was an independent predictive factor for increased OS in patients with ICC. This might be explained by that (a) circ-SMARCA5 was negatively associated with ECOG performance score, TNM stage, and abnormal CA199 status in patients with ICC, which were known as predictive factor for ICC prognosis. Therefore, circ-SMARCA5 might lead to desirable prognosis via

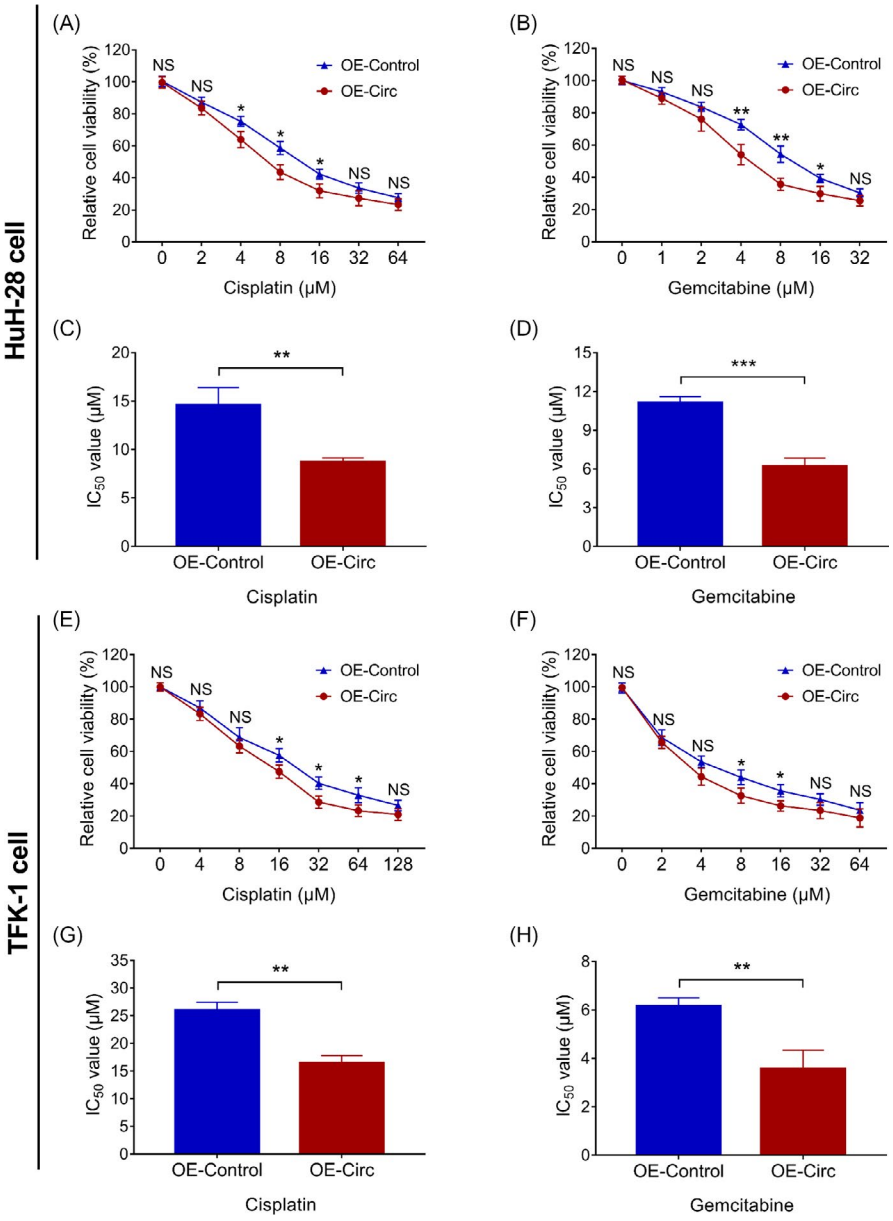

FIGURE 4 Circ-SMARCA5 increased chemotherapy sensitivity to cisplatin/gemcitabine in ICC cells after transfection. Comparison of relative cell viability between OE-Control group and OE-Circ group treated by 0, 2, 4 8, 16, 32, and 64 μmol/L cisplatin (A) and gemcitabine (B) in HuH-28 cells. Comparison of IC_{50} value of cisplatin (C) and gemcitabine (D) between OE-Control group and OE-Circ group in HuH-28 cells. Comparison of relative cell viability between OE-Control group and OE-Circ group treated by 0, 4, 8, 16, 32, 64, and 128 μmol/L cisplatin (E) and gemcitabine (F) in TFK-1 cells. Comparison of IC_{50} value of cisplatin (G) and gemcitabine (H) between OE-Control group and OE-Circ group in TFK-1 cells. ICC, intrahepatic cholangiocarcinoma; circ-SMARCA5, circular RNA SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 5; OE-Circ group, the cells transfected with circ-SMARCA5 overexpression plasmids; OE-Control group, the cells transfected with control overexpression plasmids; IC_{50}, drug concentration required to inhibit growth by 50%

interaction with these clinical-pathological tumor features. (b) Circ-SMARCA5 might be associated with SMARCA5-related functions (including DNA repair and protection against the adverse effects of DNA damage) via activating DNA damage response, contributing to improved chemosensitivity, which was validated in our further functional experiments that circ-SMARCA5 increased cell chemotherapy sensitivity to cisplatin and gemcitabine in ICC. Thus, patients with circ-SMARCA5 high expression had increased survival in a long-term period compared with those with circ-SMARCA5 low expression.[30]

Existing evidence demonstrates that circ-SMARCA5 is not simply by-products of gene splicing, but is of functionality and involved in regulating cell activities in various cancers.[11,12,15,29] For example, in cervical cancer, upregulation of circ-SMARCA5 inhibits cell proliferation, migration, and invasion, and caused cell cycle arrest via being miR-620 sponge, suggesting the anti-tumor properties of circ-SMARCA5.[12] In addition, circ-SMARCA5 promotes the expression of an anti-tumor gene, TIMP3, via functioning as the sponge of miR-181b and miR-17, resulting in inhibitory effect on HCC cell proliferation and migration.[15] However, the regulatory function of circ-SMARCA5 on cell activities in ICC has not been investigated yet. We found that upregulation of circ-SMARCA5 suppressed ICC cell proliferation and improved cell chemotherapy sensitivity to cisplatin as well as gemcitabine. The possible reasons might include that (a) upregulation of circ-SMARCA5 might activate the expressions of anti-tumor genes (such as TIMP3), which inhibited cell proliferation in ICC. (b) According to the previous study, cisplatin and gemcitabine are accepted as the standard chemotherapy regimen for ICC, and their abilities include involvement in DNA repair mechanisms, inducing cancer cell apoptosis, and inhibitory effect in DNA damage.[2,31] Furthermore, circ-SMARCA5 might boost the DNA repair ability and acquire resistance to DNA damaging agents via its located gene (SMARCA5), which reduced the formation of the drug resistance.[30] Thus, circ-SMARCA5 upregulation might enhance the chemosensitivity (cisplatin and gemcitabine) in ICC cells. Combining the findings of cellular experiments

and clinical research in our study, circ-SMARCA5 is suggested to be a candidate prognostic biomarker in ICC.

There were still some limitations in our study. (a) The potential molecule mechanism of circ-SMARCA5 in ICC was not included in our present study; therefore, further cellular experiments were needed. (b) Since we included the patients whose ages were 18 years ≤ age ≤ 80 years, therefore the result of our study might not be suitable for all the patients with ICC, and further study were needed for validation. (c) Our study was a single-centered study with a small sample size, which might lead to relatively low statistical significance. Therefore, study with larger sample size from multiple regions was needed for validation in the future.

In conclusion, circ-SMARCA5 is downregulated in tumor tissues and correlates with favorable clinicopathological features and survival profile in patients with ICC, and its upregulation not only inhibits proliferation, but also increases chemotherapy sensitivity to cisplatin and gemcitabine in ICC cells.

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How to cite this article: Lu Q, Fang T. Circular RNA SMARCA5 correlates with favorable clinical tumor features and prognosis, and increases chemotherapy sensitivity in intrahepatic cholangiocarcinoma. *J Clin Lab Anal*. 2020;34:e23138. https://doi.org/10.1002/jcla.23138