COCH predicts survival and adjuvant TACE response in patients with HCC

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Abstract. The aim of the present study was to measure the expression of Cochlin (COCH) and analyze its association with survival, recurrence and the benefits from adjuvant transarterial chemoembolization (TACE) in patients with hepatocellular carcinoma (HCC) following hepatectomy. Patients with high COCH expression levels had a poorer prognosis in terms of overall and disease-free survival rate compared with those with low COCH expression levels. Further analysis revealed that patients with low COCH expression who received TACE experienced markedly lower early recurrence rates compared with those who did not receive TACE. However, patients with high COCH expression with and without adjuvant TACE after resection experienced no difference in disease recurrence rates. The expression of COCH was found to be associated with hepatitis B virus infection, portal vein tumor thrombosis and Barcelona Clinic Liver Cancer stage in HCC. Therefore, the findings of the present study indicated that clinical detection of COCH expression may help estimate the prognosis of patients with HCC, as well as determine whether to administer TACE after surgery to prevent recurrence.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer worldwide and the cause of 8.2% of cancer-related fatalities in 2008 (1). Hepatectomy remains the first option for patients with HCC, while non-surgical approaches, such as chemotherapy, radiation, radiofrequency ablation (RFA), transarterial chemoembolization (TACE) and percutaneous ethanol injection, have been used to inhibit tumor progression and recurrence (2). Due to inadequate resection, unprecedented tumor formation or intrahepatic metastases that were not detected during resection, the majority of patients experience recurrence within 5 years of surgery (3).

TACE is globally performed as an effective treatment for HCC, as it inhibits residual tumor growth, suppresses metastasis, prevents relapse and prolongs patient survival time (4). The patients with large HCC tumor size, Child Pugh A/B or intrahepatic metastases are considered as candidates for receiving TACE 1-2 months after resection (5,6). However, not all patients are suitable candidates for TACE, as it can also result in a deterioration of liver function and prognosis after surgery (7). Individual analysis of the molecular mechanism, prediction of the effects of different treatments and selection of the most appropriate treatment based on the histological characteristics is the main focus of personalized and precision medicine (8).

Cochlin (COCH) is a secreted protein identified in glaucomatous but not normal trabecular meshwork, that has been shown to be responsive to altered fluid shear dynamics (9). COCH is mainly detected in the normal inner ear and its mutation has been found to be associated with hearing loss, glaucoma and DFNA9 (an autosomal dominant cause of non-syndromic adult-onset sensorineural hearing loss with associated variable vestibular dysfunction), while it is expressed at lower levels in the eye, spleen, cerebellum, lung, brain and thymus (10-12). Through RNA-seq analysis of 20 HCC tissues and adjacent non-neoplastic tissues, we observed that COCH was highly expressed in HCC samples (preliminary research, data not shown). However, to the best of our knowledge, whether COCH is associated with the tumorigenesis and progression of HCC has not been reported to date. Therefore, the aim of the present study was to investigate the prognostic value of COCH and its association with the effects of TACE in patients with HCC.

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Materials and methods

Patients and tissue samples. A total of 135 patients with HCC were recruited from the Shanghai Eastern Hepatobiliary Surgery Hospital (Shanghai, China) between January 2005 and December 2007. All the patients underwent hepatectomy with or without postoperative TACE. The tumor tissues were embedded in paraffin and underwent tissue microarray (TMA) analysis. The patients were selected according to the following inclusion criteria: World Health Organization performance status 0-1, Child-Pugh class A, absence of ascites, no chemotherapy or radiotherapy prior to curative resection, and confirmation of HCC diagnosis by pathological examination (13,14). The following histological features were examined: Thin beam, thick beam or pseudoglandular duct, the degree of differentiation, the degree of necrosis and infiltration, cell type and microvascular invasion. Hepatectomy was performed as previously described, Tumor-Node-Metstasis (TNM) stage was then determined (5,15). Tumor tissue, adjacent non-neoplastic tissues and non-neoplastic distant tissues were collected after hepatectomy. The tissues outside the capsule (distance ≤1 cm) were defined as adjacent non-neoplastic tissues, while the tissues outside the capsule (distance >1 cm) were defined as non-neoplastic distant tissues. The protocol of the present study was approved by the Ethics Committee of Shanghai Eastern Hepatobiliary Surgery Hospital. Patients provided written informed consent for the publication of any associated data and accompanying images.

Adjuvant TACE. Patients received hepatic arterial angiography and adjuvant TACE within 1-2 months of hepatectomy. Patients without a tumor in the residual liver received preventive TACE (10 mg hydroxycamptothecin, 20 mg pirarubicin and 1 ml lipiodol). Patients with tumor in the residual liver received therapeutic TACE (10 mg hydroxycamptothecin, 20 mg pirarubicin, 100 mg oxaliplatin and 5 ml Lipiodol). Positron emission tomography-computed tomography (PET-CT) or magnetic resonance imaging evaluation was performed 1 month after the treatment, in order to decide whether subsequent TACE treatment should be performed.

Follow-up. The follow-up visits took place once every 3-6 months in the first 5 years after surgery. A complete physical examination was performed at each follow-up visit. Serum α-fetoprotein measurements, liver function tests and an abdominal ultrasound were performed. Furthermore, PET-CT or magnetic resonance imaging was performed upon suspicion of recurrence or metastasis. Patients with recurrence received repeat hepatectomy, chemotherapy, radiotherapy or local ablative therapy, depending on the size, location and number of recurrent tumors, as well as the liver function. Overall survival (OS) time was defined as the time from hepatectomy to the date of death or the date of the last follow-up. Disease-free survival (DFS) time was defined as the time from hepatectomy to recurrence or the date of the last follow-up.

TMA and immunohistochemical analysis. The clinical tissue samples were fixed with 10% formaldehyde at room temperature for 24 h and embedded in paraffin. The section thickness was 3-5 μm. Hematoxylin and eosin staining (room temperature for 50 sec for both) was performed on the tumor tissues, adjacent non-neoplastic tissues and non-neoplastic distant tissues to determine optimal contents. Tissue samples (1 mm in diameter) were punched from paraffin-embedded tissues and then arranged in a TMA module with 0.2-mm intervals (Shanghai Biochip Company, Ltd.). An immunohistochemical assay was performed as previously reported (16). The antibody against COCH was purchased from Abcam (cat. no. ab171410; 1:100 dilution). Secondary antibody was purchased from Agilent Technologies, Inc. (anti-Rabbit-HRP; cat. no. K400311-2; 1:100 dilution). Stained sections were evaluated by three different researchers who were blinded to the clinical characteristics. The immunohistochemical staining intensity was scored as follows based on the coloration intensity and the percentage of stained cells: The staining intensity was score as: 0, negative; 1, weak; 2, moderate; or 3, strong. The percent positivity was scored as 0-100%. The percentage and staining intensity scores were multiplied to yield immunoreactive score: Scores of 0 or 1 were defined as low expression of COCH, while scores of 2 and 3 were defined as high expression of COCH (17). Cases in which there were disagreements on the immunohistochemistry staining intensity score were discussed with other researchers until a consensus was reached.

RNA collection, cDNA synthesis and reverse transcription-quantitative(RT-q)PCR analysis. Total RNA was extracted from cell lines, fresh-frozen tumor specimens and healthy control tissues using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.). cDNA synthesis was performed using random hexamers (Roche Diagnostics) and SuperScriptII reverse transcription (Invitrogen; Thermo Fisher Scientific, Inc.). RT-qPCR was performed using an ABI 7900 Fast Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) and SYBR Green PCR kit (Takara Bio, Inc.). The temperature protocol of RT-PCR was: 95˚C for 1 min, 35 cycles (95˚C for 30 secs, 60˚C for 30 secs and 72˚C for 30 secs). 18S was qualified as reference gene (18). The primer sequences were as follows: COCH, forward: 5'-AGAAAACACCCGAGAAGAAAACT-3' and reverse: 5'-CCAATTCCCAACATTAGAGCCA-3' and 18S, forward: 5'-CGGCTACGACATCCAAGGAA-3' and reverse: 5'-GCTGGAATTAGCGCGGCT-3'. The quantity of mRNA was determined using the $2^{-\Delta\Delta Cq}$ method (19).

Statistical analysis. All the statistical analyses were conducted using SPSS version 20.0 (IBM Corp.). The differences between tumor tissues, adjacent non-neoplastic tissues and non-neoplastic distant tissues were determined by Kruskal-Wallis test, followed by Dunn's test. The associations between COCH expression and clinical data were determined using the χ^2 test (once expected values were ≤5, Fisher's exact test was chosen). The differences in OS and DFS times between groups were determined by Kaplan-Meier analysis with log-rank tests. A univariate analysis was performed to determine the variants with statistical significance. The Cox regression model was used to analyze the effect of independent factors on OS and DFS time, based on the variants selected by univariate analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient histological characteristics. The characteristics of the patients (n=135) are summarized in Table I. All the patients were diagnosed through radiological and pathological examination, and had undergone hepatectomy, with or without TACE. Reverse transcription-PCR analysis of 27 patients revealed that the mRNA level of COCH was higher in the tumor tissues compared with that in the adjacent and distant non-neoplastic tissues (Fig. 1A). The patients were divided into two groups according to the expression of COCH, which was determined by the immunostaining intensity of TMA slides (Fig. 1B). The immunostaining results were analyzed and evaluated by three individual researchers independently.

High COCH expression predicts a poor prognosis of HCC. In all patients, COCH expression levels were found to be significantly associated with portal vein tumor thrombosis (PVTT; P=0.039) and BCLC stage (P=0.049) (Table II). Kaplan-Meier analysis revealed that patients with high COCH expression exhibited a markedly shorter OS time compared with patients with low COCH expression (high-COCH patients: Median OS time, 12.208 months; 95% confidence interval (CI), 9.000-15.415; and low-COCH patients: Median OS time, 24.259 months; 95% CI, 18.218-30.389; P<0.001; Fig. 1C). Furthermore, patients with high COCH expression exhibited earlier recurrence of HCC (high-COCH patients: Median DFS time, 9.371 months; 95% CI, 5.085-13.657; and low-COCH patients: Median DFS time, 18.388 months; 95% CI, 11.981-24.769; P=0.010; Fig. 1D).

COCH expression level may predict the effect of adjuvant TACE. The aim of adjuvant TACE is mainly to prevent HCC recurrence. As shown in Fig. 2A and B, adjuvant TACE prolonged the OS (adjuvant TACE group: Median OS time, 25.647 months; 95% CI, 19.250-32.044; and control group: Median OS time, 12.396 months; 95% CI, 9.045-15.693; P<0.001; Fig. 2A) and 5-year DFS (adjuvant TACE group: Median DFS time, 19.836 months; 95% CI, 13.250-26.422; and control group: Median DFS time, 10.103 months; 95% CI, 5.211-14.994; P<0.001; Fig. 2B) times of the patients.

The results shown in Fig. 1 indicated that COCH predicted a poor patient prognosis and early cancer recurrence. The present study also investigated the association between COCH expression and the effectiveness of TACE. TACE treatment did not decrease the recurrence rate of patients with high COCH expression compared with that of patients who did not receive TACE (control group vs. TACE group: Median DFS time, 8.254 months vs. 12.402 months; 95% CI, 2.725-13.782 vs. 4.915-19.890; P=0.087; Fig. 3B). However, patients with low COCH expression exhibited a significantly lower recurrence rate after TACE (low COCH group vs. high COCH group: Median DFS time, 27.348 months vs. 12.386 months; 95% CI, 17.310-37.385 vs. 3.895-20.878; P=0.002; Fig. 3D). As recurrence significantly affects the prognosis of patients with HCC, the OS time of patients in different COCH expression groups, with and without TACE, was analyzed. TACE treatment was found to not be suitable for patients with high COCH expression, as it did not reduce recurrence or prolong OS time (control group vs. TACE group: Median OS time, 11.000 months vs. 14.214 months; 95% CI, 7.574-14.426 vs. 7.923-20.505; P=0.485; Fig. 3A). However, TACE improved the OS time of the HCC patients with low COCH expression (control group vs. TACE group: Median OS time, 39.565 months vs. 14.200 months; 95% CI, 30.422-48.708 vs. 7.947-20.453; P<0.001; Fig. 3C).

Table I. Patient characteristics (n=135).

Characteristic	Value
Age, years	
Mean ± SD	48.8±10.294
Median (range)	49 (26-75)
Sex, n	
Male	118 (87.4)
Female	17 (12.6)
HBsAg, n	
Positive	126 (93.3)
Negative	9 (6.7)
Largest tumor size, cm	
≤5	21 (15.6)
>5	114 (84.4)
Serum AFP, ng/ml	
≤400	34 (25.2)
>400	101 (74.8)
Tumor number, n	
Single	122 (90.4)
Multiple	13 (9.6)
Portal vein tumor thrombus, n	
Negative	44 (32.6)
Positive	91 (67.4)
Tumor capsule, n	
Complete	39 (28.9)
Incomplete	96 (71.1)
BCLC stage, n	
A	13 (9.6)
B	31 (23)
C	91 (67.4)
TNM stage, n	
I/II	30 (22.2)
III/IV	105 (77.8)
Adjuvant TACE, n	
Yes	39 (28.9)
No	96 (71.1)

AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HBsAg, hepatitis B surface antigen; TACE, transarterial chemoembolization; TNM, Tumor-Node-Metastasis.

Univariate and multivariate analysis of prognostic factors. Cox regression analysis was employed to analyze the association between COCH level and the effects of TACE. As shown in Table III, univariate Cox regression analysis indicated that adjuvant TACE, the size and number of tumors, completeness

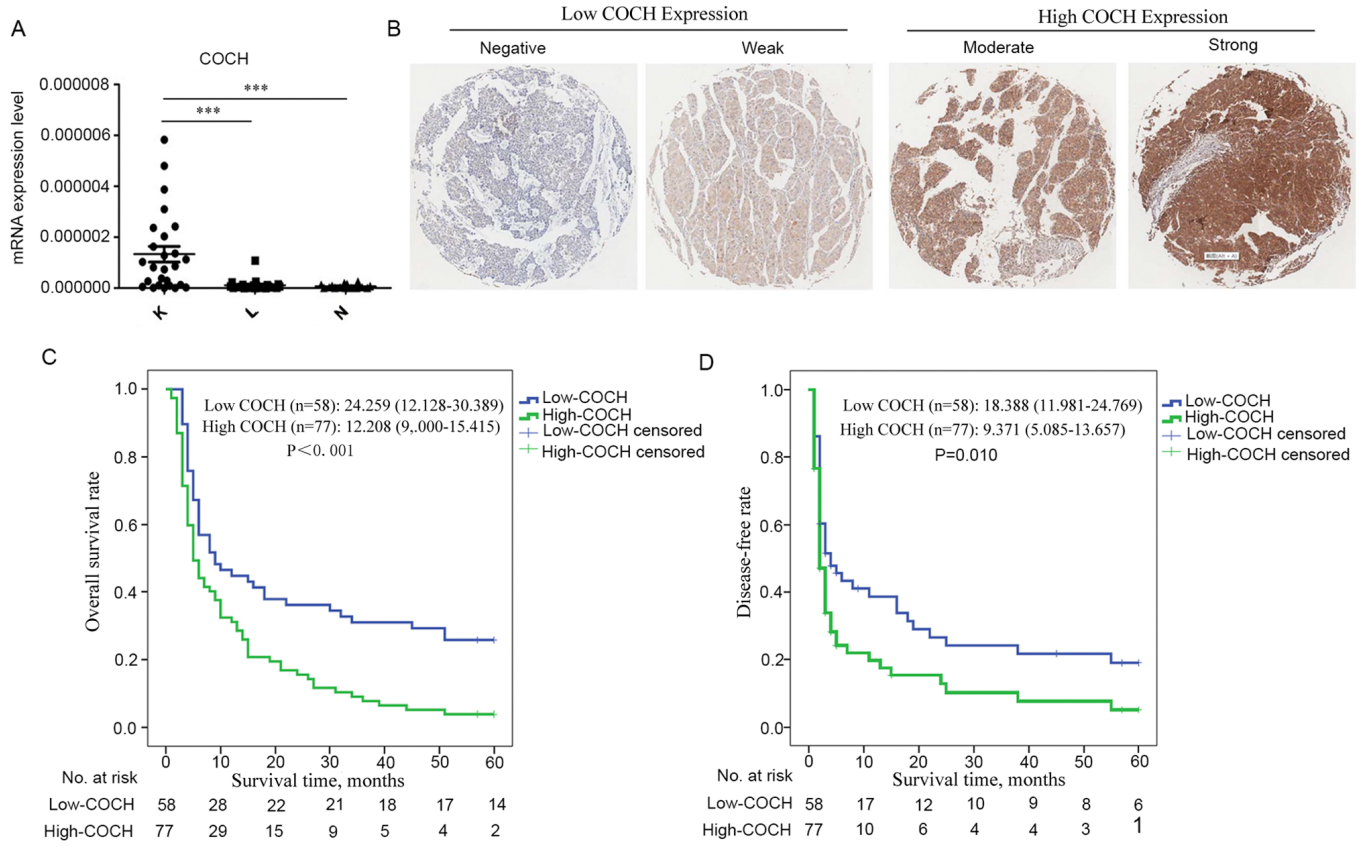

Figure 1. COCH expression is associated with OS and DFS. (A) mRNA level of COCH in tumor tissues, adjacent non-neoplastic tissues and distant non-neoplastic tissues from 27 patients with HCC. ***P<0.05. (B) Immunohistochemical analysis of COCH in patients with HCC. (C) Kaplan-Meier analysis of OS in patients with HCC and different COCH expression levels. (D) Kaplan-Meier analysis of DFS in patients with HCC and different COCH expression levels. COCH, cochlin; HCC, hepatocellular carcinoma; OS, overall survival; DFS, disease-free survival; K, tumor tissues; L, adjacent non-neoplastic tissues; N, distant non-neoplastic tissues.

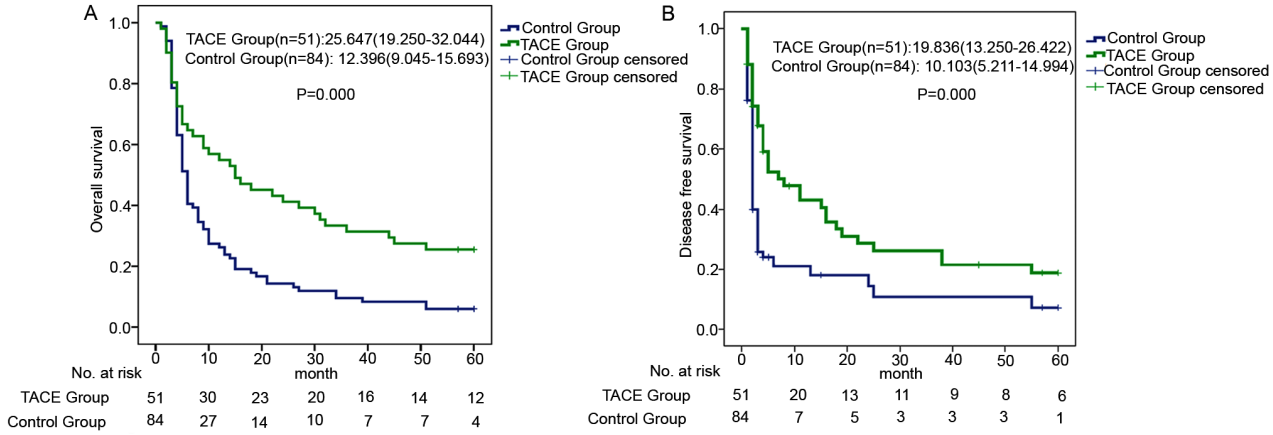

Figure 2. Prognostic significance of postoperative adjuvant TACE. (A) Kaplan-Meier analysis of the overall survival in patients with and without TACE. (B) Kaplan-Meier analysis of the 5-year disease-free survival in patients with and without TACE. TACE, transarterial chemoembolization.

of the tumor capsule and HBV infection (shown as HBsAg in the table) were associated with recurrence in patients with low COCH expression. Multivariate Cox regression analysis based on the factors identified as statistically significant on the univariate Cox regression analysis revealed that TACE was an independent biomarker for 5-year DFS in HCC patients with low COCH expression (hazard ratio, 0.4727; 95% CI, 0.3503-2.139; P=0.0324). In addition, tumor number (P=0.0033) and tumor size (P=0.0393) were independent predictors of 5-year DFS. However, the univariate analysis revealed that no variable was significantly associated with tumor recurrence in patients with high COCH expression.

Discussion

Partial hepatectomy is the recommended first-line treatment for primary HCC. However, the local recurrence rate in the first 5 years following resection is as high as 70% (20). Satellite

Table II. Association between COCH protein expression and clinicopathological characteristics.

Characteristic	COCH expression in HCC			P-value
	Total, n	Low, n (%)	High, n (%)	
Total patients	135	58 (42.96)	77 (57.03)	
Age, years				
≤49	69	30 (22.22)	39 (28.89)	0.902
>49	66	28 (20.74)	38 (28.15)	
Sex				
Male	118	49 (36.30)	69 (51.11)	0.374
Female	17	9 (0.07)	8 (0.06)	
HBsAg				
Negative	9	1 (0.01)	8 (0.06)	0.077[a]
Positive	126	57 (42.22)	69 (51.11)	
Serum AFP, ng/ml				
≤400	34	18 (13.33)	16 (11.85)	0.174
>400	101	40 (29.63)	61 (45.18)	
Largest tumor size, cm				
≤5	21	10 (0.07)	11 (0.08)	0.639
>5	114	48 (35.56)	66 (48.89)	
Tumor capsule				
Complete	39	15 (11.11)	24 (17.78)	0.501
Incomplete	96	43 (31.85)	53 (39.26)	
Portal vein tumor thrombus				
Positive	92	34 (25.19)	58 (42.96)	0.039[b]
Negative	43	24 (17.78)	19 (14.07)	
Tumor number				
Single	122	54 (0.40)	68 (0.50)	0.350
Multiple	13	4 (0.03)	9 (0.07)	
BCLC stage				
A	13	8 (0.06)	5 (0.04)	0.049[b]
B	31	16 (0.12)	15 (11.11)	
C	91	34 (21.16)	57 (42.22)	
TNM stage				
I/II	30	17 (12.59)	13 (0.10)	0.086
III/IV	105	41 (30.37)	64 (47.41)	

[a]Value obtained using Fisher's exact test; [b]P<0.05; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HBsAg, hepatitis B surface antigen; TACE, transarterial chemoembolization; TNM, Tumor-Node-Metastasis; COCH, cochlin; HCC, hepatocellular carcinoma.

lesions, cirrhosis and tumor size are considered to be closely associated with postoperative recurrence (21). Several treatment options may be used to prevent the recurrence of HCC, including repeat hepatectomy, RFA and TACE (3). RFA and TACE may be considered more suitable for patients with Child-Pugh grade A or B, and for those with a greater size or number of tumors (22). However, based on the heterogeneity of HCC, not all patients will benefit from TACE. Patients with large tumors or venous invasion are at higher risk of recurrence and are advised to receive TACE in clinical practice (23). Molecular analysis of *in situ* and recurrent tumors may improve our understanding of the mechanisms underlying recurrence and help identify prognostic biomarkers (24,25). Several systematic analyses based on >10,000 patients with HCC demonstrated that patients receiving TACE experienced a survival benefit compared with the control group (26,27). However, other studies have reported different results regarding recurrence and survival after receiving TACE. A Cochrane analysis of 6 trials observed no superior effectiveness of TACE compared with the control group (28,29). This controversy focuses not only on patient recruitment for TACE, but also on the need for more large-scale trials (30,31). Therefore, patient selection is crucial when considering TACE.

The present study demonstrated that COCH was a suitable predictor of survival and recurrence in patients with HCC. The expression of COCH was closely associated with PVTT,

Table III. Univariate and multivariate Cox regression analyses of 5-year disease-free survival in patients with different COCH expression levels.

	Low COCH expression		High COCH expression	
Variables	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Univariate analysis				
Adjuvant TACE (yes vs. no)	0.386 (0.198-0.753)	0.005[a]	0.632 (0.365-1.093)	0.101
Age (>49 years vs. ≤49 years)	1.429 (0.772-2.646)	0.256	0.954 (0.575-1.583)	0.857
Sex (male vs. female)	0.669 (0.263-1.704)	0.399	0.821 (0.352-1.913)	0.647
HBsAg[*] (negative vs. positive)	21.931 (0.016-29732.5)	0.401	1.308 (0.562-3.043)	0.534
Serum AFP (>400 ng/ml vs. ≤400 ng/ml)	1.326 (0.678-2.596)	0.41	1.192 (0.640-2.222)	0.580
Largest tumor size (>5 cm vs. ≤5 cm)	2.903 (1.124-7.497)	0.028[a]	2.044 (0.919-4.546)	0.080
Portal vein tumor thrombus (negative vs. positive)	1.173 (0.634-2.170)	0.611	1.593 (0.869-2.919)	0.132
Tumor capsule (complete vs. incomplete)	0.440 (0.202-0.959)	0.039[a]	0.749 (0.430-1.303)	0.306
Tumor number (single vs. multiple)	4.035 (1.364-11.937)	0.012[a]	0.882 (0.401-1.942)	0.755
BCLC stage (A vs. B vs. C)	1.292 (0.851-1.960)	0.229	1.474 (0.951-2.284)	0.083
TNM (I+II vs. III+IV)	1.513 (0.759-3.015)	0.239	1.753 (0.856-3.590)	0.125
Multivariate analysis				
Adjuvant TACE (yes vs. no)	0.4727 (0.3503-2.319)	0.0324[a]	NA	NA
Largest tumor size (>5 cm vs. ≤5 cm)	2.7752 (0.4953-2.061)	0.0393[a]	NA	NA
Tumor capsule (complete vs. incomplete)	0.4576 (0.4044-1.933)	0.0532	NA	NA
Tumor number (single vs. multiple)	5.3590 (0.5713-2.939)	0.0033[a]	NA	NA

[*]Means HBV infection. [a]P<0.05. AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HBsAg, hepatitis B surface antigen; TACE, transarterial chemoembolization; TNM, Tumor-Node-Metastasis; COCH, cochlin.

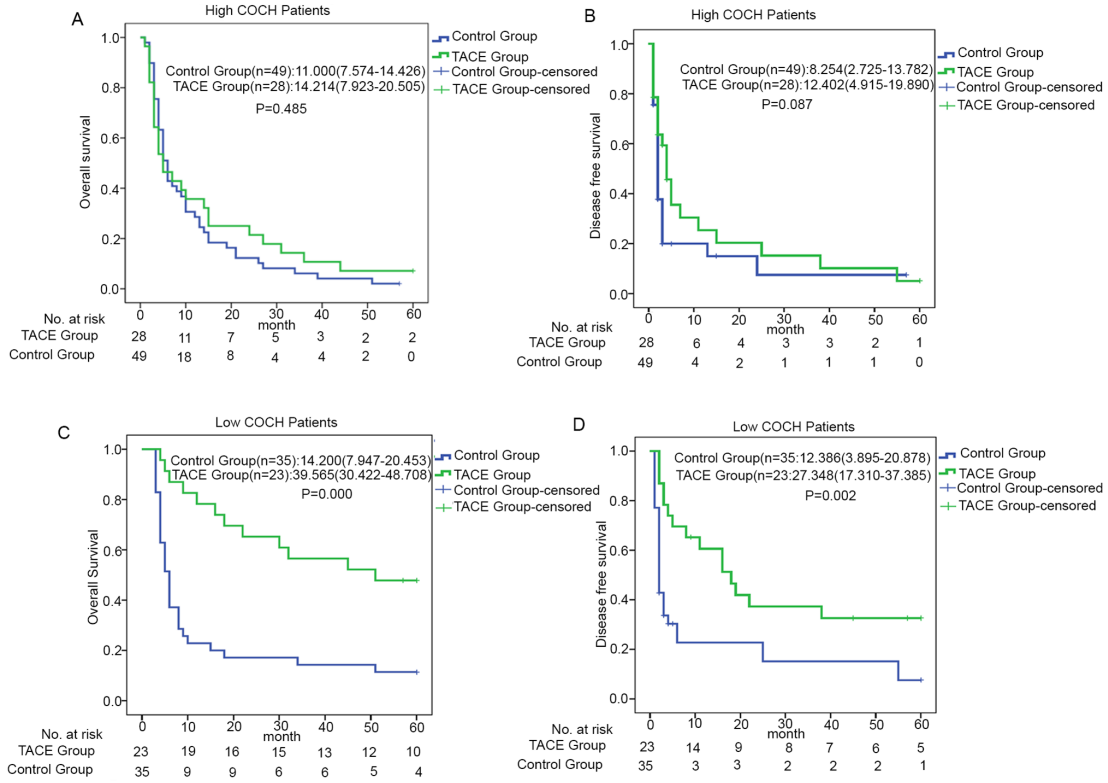

Figure 3. Prognostic value of COCH for postoperative adjuvant TACE efficacy. (A) Kaplan-Meier analysis of the association between adjuvant TACE therapy and OS in patients with HCC and high COCH expression. (B) Kaplan-Meier analysis of the association between adjuvant TACE therapy and 5-year DFS in patients with HCC and high COCH expression. (C) Kaplan-Meier analysis of the association between adjuvant TACE therapy and OS in patients with HCC and low COCH expression. (D) Kaplan-Meier analysis of the association between adjuvant TACE therapy and 5-year DFS in patients with HCC and low COCH expression. COCH, cochlin; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; OS, overall survival; DFS, disease-free survival.

HBV infection and BCLC stage. However, when the efficacy of adjuvant TACE was analyzed, only patients with low COCH expression appeared to benefit from the treatment. To the best of our knowledge, the present study is the first to report that COCH is associated with recurrence and may be useful in evaluating prognosis. It may also serve as a factor determining whether TACE should be administered to prevent recurrence and prolong OS time. Measuring COCH expression may also help evaluate the effect of TACE following hepatectomy and to determine whether to select TACE as a first-line adjuvant therapy. However, the univariate analysis in patients with high COCH expression revealed that the recurrence rate was not associated with any variables, including BCLC and TNM stage, which was reported to be associated with recurrence (3,32). This discrepancy may be due to the limited number of included patients. A larger study is required to confirm the results of the present study.

The mechanisms underlying the beneficial effect of TACE treatment on patients with low COCH expression remain elusive. The results of immunohistochemical analysis revealed that COCH was expressed in both the nucleus and the cytoplasm. It has not yet been reported whether COCH is more highly expressed in HCC and whether its expression is associated with the survival and recurrence of HCC, but the expression of COCH in normal liver tissue is low (33). TACE inhibits recurrence mainly by suppressing the early metastasis of tumor cells (34). However, it is difficult to detect the small intrahepatic metastases that contribute to early tumor recurrence, before or after hepatectomy. Theoretically, therapies focusing on undetected intrahepatic metastases are crucial for preventing the recurrence of HCC. However, some studies highlight the need for the careful selection of patients for TACE, as the treatment may damage liver cells and compromise liver function, which is important to help optimize the benefit of the overall HCC treatment course (7,35). The side effects of TACE may affect patient survival, which may explain why patients with high COCH expression do not benefit from TACE treatment.

In the present study, COCH was identified as a potential biomarker of HCC prognosis. Patients with high COCH expression exhibited poor OS times, early recurrence and no obvious response to adjuvant postoperative TACE. By contrast, patients with low COCH expression exhibited better OS and DFS times, as well as a better response to TACE. However, the predictive value of COCH for the clinical selection of TACE usage requires further verification by large-scale clinical trials, and the underlying mechanism must also be further investigated.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CW performed the experiments and performed the statistical analysis. ZWD and CGZ collected the pathological data of the patients and prepared the tissue microarray. SW analyzed revised the clinical data and the manuscript. ZHL and ZMZ performed the molecular experiments. JP and JW designed and conceived the experiment and confirmed the authenticity of the raw data. CY conceived the study and wrote the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

HCC samples were obtained from the Shanghai Eastern Hepatobiliary Surgery Hospital, and studies on human tissues were approved by the Ethics Board of the Shanghai Eastern Hepatobiliary Surgery Hospital. Written consent was obtained from all patients.

Patient consent for publication

Patients provided written informed consent for the publication of any associated data and accompanying images.

Competing interests

The authors declare that they have no competing interests.

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