BIRC7 and STC2 expression is associated with tumorigenesis and poor outcome in extrahepatic cholangiocarcinoma

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Abstract

Background: Extrahepatic cholangiocarcinoma (EHCC) is a highly aggressive epithelial malignancy and has a poor prognosis for their insensitivity to therapies and difficulty in detection. Novel targets and biomarkers are urgently needed to develop for functional, diagnostic and prognostic application on EHCC.

Methods: 100 EHCC tissues、30 peritumoral tissues, 10 adenoma and 15 normal biliary tract tissues were assayed using EnVision immunohistochemistry.

Results: The expression of BIRC7 and STC2 proteins were higher in EHCC tissues than those in peritumoral tissues, adenoma and normal biliary tract tissues. The positive rates of BIRC7 and STC2 expression were significantly higher in cases with lymph node metastasis, invasion to surrounding tissues/organs, and TNM stage III and/or IV and unable to undergo resection (biopsy only). Kaplan-Meier survival curves demonstrated that the overall survival of positive-BIRC7 or positive-STC2 patients was significantly lower than those of negative-BIRC7 or negative-STC2, respectively. Cox-proportional regression analysis demonstrated that positive-BIRC7 and positive-STC2 expression, along with poor differentiation of EHCC, tumor size >3cm, lymph node metastasis, invasion to surrounding tissues/organs and unable to undergo resection are independent prognostic factors of EHCC patients.

Conclusions: BIRC7 and STC2 are involved in the tumorigenesis and progression of EHCC, and positive expression of BIRC7 and STC2 are associated with poor prognosis in patients with EHCC. BIRC7 and STC2 might be a potential tumor biomarker for EHCC diagnosis and prognosis.

Background

Cholangiocarcinoma (CCA) is a highly aggressive epithelial malignancy, representing 10–25% of primary hepatic malignancies worldwide[1–3]. It is defined anatomically as intrahepatic CCA (IHCC) and extrahepatic CCA (EHCC), the EHCC is further characterized as perihilar CCA, originating at the bifurcation of the common hepatic duct, or tumour of the distal bile duct. The diagnosis of EHCC is complex and is made with a combination of appropriate clinical suspicion, imaging techniques, endoscopic techniques, and cytopathological examination. Although there have been advances in the diagnosis and management of EHCC, these cancers remain challenging to treat because of their insensitivity to conventional therapies and inability to detect early tumor formation[4, 5]. In clinical practice, the most widely studied and used tumor marker is carbohydrate antigen 19 – 9 (CA19-9), however, CA19-9 has a wide variation in sensitivity (50%-90%) and specificity (54%-98%) and is often falsely elevated in benign biliary disease and/or cholangitis, with levels falling after relief of biliary obstruction and sepsis[6, 7]. Novel targets and biomarkers involved in the tumorogenesis and metastasis, diagnosis and prognosis of the EHCC are urgently needed.

Baculoviral IAP repeat-containing 7 (BIRC7), also known as Livin, encodes a member of the inhibitor of apoptosis protein (IAP) family, and contains a single copy of a baculovirus IAP repeat (BIR) as well as a RING-type zinc finger domain[8, 9]. The BIR domain has a well-characterized role in interacting with caspases and inhibiting apoptosis, while the RING finger domain sometimes enhances antiapoptotic activity but does not inhibit apoptosis alone[9, 10]. A series of previous studies have showed that aberrant BIRC7 expression is closely related to the occurrence, progress, clinical biological behaviors and prognosis of in various human

cancer tissues, including melanoma, breast, colon, prostate cancer and hepatocellular carcinoma [11–13]. BIRC7 up-regulation is mainly a risk factor for cancer progression, poor prognosis and resistance to chemotherapy and radiotherapy [14, 15]. It might serve as a valuable biomarker of increased recurrence risk in prostate cancer [16]. Moreover, it has been shown that down-regulation of BIRC7 expression resensitizes tumor cells to apoptosis and chemotherapy [15, 17] and leads to tumor volume reduction in a xenograft model of colorectal cancer [18]. In short, BIRC7 might represent a new potential biomarker and target for future diagnosis and treatment of tumor.

Stanniocalcin 2 (STC2) encodes a secreted, homodimeric glycoprotein that is expressed in a wide variety of tissues and have autocrine or paracrine functions [19]. STC2 and its homologues, STC1, may play a role in cellular calcium/phosphate homeostasis [20]. Previous studies have revealed that STC2 plays an oncogenic role in many human cancers [21–27]. The differential expression levels of STC2 have certain guiding significance for the prediction, metastasis and prognosis of gastric cancer [24], colorectal cancer [22], prostate cancer [23], breast cancer[21], laryngeal squamous cell carcinomaand [25], head and neck squamous cell carcinoma[26], endometrial cancer [27], ect. Moreover, recent studies have found that STC2 protein expression may be a valuable biomarker for cancer progression, as well as a prognostic marker for poor outcome [24–27].

Little is currently known about the role of BIRC7 and STC2 in EHCC. The aim of this study was to investigate the clinicopathological significance and prognostic values of BIRC7 and STC in a large cohort of EHCC by immunohistochemistry.

Methods

Case selection

Between Jan 2001 and Dec 2013, 100 EHCC tissue, 30 peritumoral tissues, 10 billiary tract adenoma and 15 normal billiary tract tissues were obtained at the Second and Third Xiangya Hospitals, Central South University. The patients who received chemotherapy or radiation therapy before surgery were ruled out in the study. All specimens were confirmed by two experienced pathologists who were blinded to all clinical data. The study protocol was approved by the ethical committee of Central South University. The follow-up time for EHCC was 30 months, and patients who survived more than 30 months were treated as censored cases. The clinic characteristics of EHCC were showed in Table 2.

The pericancerous tissues were collected from 30 cases of EHCC who underwent surgery, including 12 cases of normal tissues,8 case of mild dysplasia，6 cases of moderately dysplasia and 4 cases of severe dysplasia. The patient age ranged from 35 to 72 (48.5 ± 9.2) years, including 20 males and 10 females. The billiary tract adenoma tissues also collected from 10 patients (6 males and 4 females) who underwent surgery. The patient age ranged from 33 to 70 (46.7 ± 10.2) years. The results of pathological examination showed 6 cases of normal tissues，2 cases of mild dysplasia and 2 cases of moderate to severe dysplasia. The normal billary tract tissues (n = 15, 9 males and 6 females) were collected from donors of liver transplantation.

Immunohistochemistry

The tissues were routinely fixed in 4% formaldehyde solution and embedded in paraffin. Rabbit anti-human BIRC7 and STC2 polyclonal antibody were obtained from Dako Corporation (Carpentaria, CA,USA). EnVisionTM Detection Kit was purchased from Dako Laboratories (CA, USA). Positive controls were provided by the EnVisionTM Detection Kit. EnVision immunohistochemistry of BIRC7 and STC2 was performed according to manufacturer's protocol. Briefly, Formalin-fixed and paraffin-embedded tissue samples were cut into 4-μm thick sections. The sections were deparaffinized and then incubated with 3% H2O2 for 15 min. The heat-induced epitope retrieval was conducted with sodium citrate buffer (10 mM Sodium citrate, 0.05%Tween 20, pH = 6.0) at 96℃ for 30 min. The sections were incubated with rabbit anti-human BIRC7 and STC2 primary antibody (1:100 dilution) for 2 hr after they were soaked in PBS for 3 × 5 min. The sections were incubated with several drops of Solution A (ChemMateTMEnVison+/HRP) for 30 min followed by DAB staining and haematoxylin counter-staining. The sections were dehydrated, soaked in xylene, and mounted with neutral balsam. Ten fields were randomly selected and five hundred cells per section were independently examined by two pathologists. Average percentage of positive cells were calculated and cases with positive cells ≥ 25% were considered positive.

Statistical analysis

Comparison between groups was performed using χ^2 test or Fisher's exact test in SPSS 17.0 (IBM Corp., USA). The overall survival of patients with EHCC was analyzed by Kaplan-Meier univariate survival analysis and log-rank tests. Cox proportional hazards regression model analysis was performed and the 95% confidence interval was calculated. Differences with p-values < 0.05 were considered statistically significant.

Results

BIRC7 and STC2 protein expression in EHCC tissues, peritumoral tissues, billiary tract adenoma and normal tissues

Immunohistochemical staining showed that positive BIRC7 (Fig. 1) and STC2 (Fig. 2) expressions were located in the cytoplasm. In the 100 EHCC tissues, 56 and 58 of them were BIRC7 (56.0%) and STC2 (58.0%) positive, respectively (Table 1). In the 30 peritumoral tissues, 9 and 10 of them were BIRC7 (30.0%) and STC2 (33.3%) positive, respectively (Table 1). In 10 adenoma tissues, 1 and 2 of them were BIRC7 (10.0%) and STC2 (20.0%) positive, respectively (Table 1). In all 15 normal tissues, the expressions of BIRC7 and STC2 were negative (Table 1). The positive rates of BIRC7 or STC2 were significantly higher in EHCC than those in peritumoral,adenoma and normal tissues ($P < 0.05$ or $P < 0.01$).

Table 1
Comparison of BIRC7 and STC2 expression in normal tissue, adenoma, peritumoral and EHCC tissues.

Tissue type	n	BIRC 7 positive (%)	STC2 positive (%)
EHCC	100	56 (56.0)	58 (58.0)
Peritumoral tissues	30	9 (30.0)*	10 (33.3)*
Adenoma	10	1 (10.0)*	2 (20.0)*
Normal tissues	15	0 (0.0)**	0 (0.0)**
*, $P < 0.05$, vs EHCC;** $P < 0.01$ vs EHCC.			

The expressions of BIRC7 and STC2 protein were associated with clinicopathological characteristics of EHCC

As shown in Table 2, increased positive expression of BIRC7 and STC2 in EHCC were significantly related with clinicopathological features of patients with lymph node metastasis, invasion to the surrounding tissues/organs, TNM stage III and/or IV and unable to undergo resection (biopsy only) ($P < 0.05$ or $P < 0.01$). The expressions of BIRC7 and STC2 exhibited no significant association with age,sex, tumor diameter and degree of differentiation ($P > 0.05$). Among the 56 cases with positive BIRC7 expression, 40 cases was positive expression of STC2. Among the 44 cases with negative BIRC7 expression, 26 cases was negative expression of STC2. The expression of BIRC7 was positively correlated with STC2 in EHCC ($\chi^2 = 9.421$, $P = 0.003$).

Table 2

Correlations of BIRC7 and STC2 protein expression with the clinicopathological characteristics of EHCC

	n	BIRC7			STC2		
		Pos (n, %)	χ^2	P value	Pos (n, %)	χ^2	P value
Age (year)							
≤45 years	17	10 (58.8)	0.066	0.797	11 (64.7)	0.378	0.539
45 years	83	46 (55.4)			47 (56.6)		
Sex							
Male	61	37 (60.7)	1.376	0.241	32 (52.5)	1.971	0.160
Female	39	19 (48.7)			26 (66.7)		
Differentiation							
Well	31	12 (38.7)	5.560	0.062	14 (45.2)	3.064	0.216
Moderately	34	21 (61.8)			22 (64.7)		
Poorly	35	23(65.7)			22 (62.9)		
Tumor size							
≤3 cm	62	32 (51.6)	1.274	0.259	35 (56.5)	0.161	0.689
3 cm	38	24 (63.2)			23 (60.5)		
Lymphnode metastasis							
No	62	26 (41.9)	13.098	0.000	25 (40.3)	20.930	0.000
Yes	38	30 (78.9)			33 (86.8)		
Invasion							
No	33	13 (39.4)	5.512	0.023	12 (36.4)	9.465	0.002
Yes	67	43 (67.2)			46 (68.7)		
TNM stage							
+	35	13 (37.1)	10.303	0.006	11 (31.4)	18.751	0.000
III	38	22 (57.9)			24 (63.2)		
IV	27	21 (77.8)			23 (85.2)		
Surgery							
Radical	54	22 (40.7)	12.097	0.002	22 (40.7)	14.371	0.001
Palliative	36	28 (77.8)			28 (77.8)		

	n	BIRC7			STC2		
		Pos (n, %)	χ^2	P value	Pos (n, %)	χ^2	P value
Biopsy	10	6 (60.0)			8 (80.0)		

BIRC7 and STC2 protein expressions correlated with overall survival in patients with EHCC

Of the 100 EHCC patients, 58 patients died within 12 months,24 patients died within 24 months, 10 patients died within 30 months, and 12 patients who survived longer than 30 months were included in the analysis as censored cases. The results revealed that the degree of differentiation, lymph node metastasis, invasion to the surrounding tissues/organs, TNM stage and surgical procedure were significantly associated with the mean overall survival time of patients of EHCC ($P < 0.05$ or $P < 0.01$) (Table 3). Kaplan-Meier survival curves demonstrated that the overall survival of positive-BIRC7 (Fig. 3A) or positive-STC2 (Fig. 3B) patients was significantly lower than those of negative-BIRC7 or negative-STC2, respectively. The results of Cox-proportional regression analysis were shown in Table 4, the data suggested that positive expressions of BIRC7 and STC2, along with poor differentiation of EHCC, tumor size > 3 cm, lymph node metastasis, invasion to surrounding tissues/organs and unable to undergo resection (biopsy only), are independent prognostic factors (Tables 4). Finally, we calculated the AUC for BIRC7 (AUC = 0.662, 95%CI: 0.574–0.750) and STC2 (AUC = 0.681, 95%CI: 0.594–0.768), respectively (Fig. 4).

Table 3
Relationships between the clinicopathological characteristics, expression BIRC7 and STC2 and mean survival of patients with EHCC

	n	Mean survival (month)	Chi-square	P-value
Sex				
Male	61	12.67(3–30)	0.001	0.980
Female	39	12.59(4–30)		
Age (year)				
≤45	17	13.82(3–30)	0.667	0.414
45	83	12.10(3–30)		
Differentiation				
Well	31	18.46(5–30)	27.655	0.000
Moderately	34	11.41(3–30)		
Poorly	35	7.97(3–30)		
Tumor size				
≤3 cm	62	12.62(3–30)	0.235	0.628
3 cm	38	12.03 (5–30)		
TNM stage				
+	35	18.57(7–30)	57.569	0.000
III	38	11.05(3–30)		
IV	27	6.26(3–13)		
Lymph node metastasis				
No	62	15.52(4–30)	39.001	0.000
Yes	38	7.18(3–25)		
Invasion				
No	33	17.52(4–30)	17.399	0.000
Yes	67	9.87 (3–30)		
Surgery				
Radical	54	16.62(3–30)	48.388	0.000
Palliative	36	7.58(4–24)		
Biopsy	10	6.90(3–14)		

	n	Mean survival (month)	Chi-square	P-value
BIRC7				
Negative	44	17.86(6–30)	23.060	0.000
Positive	56	9.07(3–28)		
STC2				
Negative	42	18.07(5–30)	22.514	0.000
Postive	58	9.22(3–28)		

Table 4
Multivariate Cox regression analysis of prognostic factors in patients with ECC

	Factors	B	SE	wald	P	RR	95%CI	
							Lower	Upper
Differentiated degree	Well/moderately/poorly	0.537	0.154	12.159	0.000	1.711	1.265	2.314
Tumor size	≤ 3 cm/>3 cm	0.530	0.248	4.567	0.033	1.699	1.045	2.762
Lymph node metastasis	No/yes	0.917	0.295	9.663	0.002	2.502	1.403	4.460
Invasion	No/yes	1.078	0.368	8.581	0.003	2.939	1.429	6.045
TNM stage	I, II/III/IV	0.756	0.264	8.200	0.004	2.130	1.269	3.573
Surgery	Radical/ Palliative/ Biopsy	0.601	0.188	10.220	0.001	1.824	1.262	2.637
BIRC7	Negative /Postive	0.912	0.289	9.959	0.002	2.489	1.413	4.386
STC2	Negative /Postive	0.740	0.283	6.837	0.009	2.096	1.204	3.650

Discussion

The late-stage detection and poor prognosis of EHCC has led to an urgent need for potential biomarkers that can be applied in clinical settings[28]. To our knowledge, this is the first study that systematically investigates the expression of BIRC7 and STC2 and their clinicopathological significances in EHCC. The study demonstrated that the rate of overall positive staining with BIRC7 and STC2 was significantly increased in EHCC tumors han those in other tissues, and positive expressions of BIRC7 and STC2 were associated with progressive clinicopathological features. The study also implied that positive-expression of BIRC7 and STC2 is a negative prognostic factor for overall survival.

Previous studies have demonstrated that BIRC7 plays a vital role in the development and progression of malignant tumors[8, 10–13, 16, 29, 30]. In the study, our results revealed that BIRC7 expression was increased

in EHCC tissues. The expression of BIRC7 was positively correlated with TNM stage, lymph node metastasis, invasion to surrounding tissues/organs and radical resection. Kaplan-Meier survival analysis showed that overall survival in patients with BIRC7-positive expression was significantly lower than that patients with BIRC7-negative expression. Cox multivariate analysis suggested that positive BIRC7 expression is an independent prognostic factor for poor prognosis in patients with EHCC. Consistent with previous studies, these results suggested that BIRC7 are involved in the tumorigenesis and progression of EHCC, and positive BIRC7 expressions were associated with poor prognosis in patients with EHCC.

BIRC7 inhibits cell apoptosis by binding to the regulators of apoptosis and promotes cell proliferation, migration and invasion[31]. Previous study revealed knockdown of BIRC7 inhibits cell growth and invasion of gastric cancer cells through inhibiting the phosphorylation of p38 and blockade of the MAPK pathway[32]. A recent study showed that BIRC7 is involved in the regulation of epithelial-mesenchymal transition (EMT) in in breast cancer, and BIRC7 promotes the progression and metastasis of breast cancer through the regulation of EMT via the p38/GSK3β pathway[30]. However, the study also revealed that BIRC7 promotes progression of breast cancer through induction of EMT and activation of AKT signaling[29]. The role of signaling pathways involved in the BIRC7-induced tumorigenesis and progression of EHCC requires further investigation.

Growing evidences indicated that STC2 protein played crucial roles in the development and progression of carcinomas[21–27]. We also examined the relationships between STC2 expression and clinicopathologic features in EHCC. The positive rates of STC2 proteins were significant higher in EHCC tumors than those in peritumoral tissues, adenoma, and normal biliary tract tissues, and the postive expression of STC2 were associated with high TNM stage, lymph node metastasis, invasion and radical resection. Kaplan-Meier analyses confirmed that patients with postive expression of STC2 had a significantly poorer survival than those with negative STC2 expression. Multivariate analysis revealed that STC2 were independent predictors of prognosis in patients with EHCC. These results suggested that STC2 is also involved in the tumorigenesis and progression of EHCC, and positive STC2 expressions were associated with poor prognosis in patients with EHCC.

STC2 has been reported to participate in many biological events of cancer development and progression, however, the underlying mechanisms remains largely unclear[24–27, 33, 34]. Yang et al.[26] demonstrated that STC2 promotes cell proliferation, tumor growth, and metastasis through the PI3K/AKT/Snail pathway in head and neck squamous cell carcinomas. Chen et al. [33] found that STC2 promotes colorectal cancer cells tumorigenesis and EMT progression through activating ERK/MEK and PI3K/AKT signaling pathways. A recent study suggested that STC2 silencing suppressed the migration of colorectal cancer cells and the occurrence of EMT which may be related to the Wnt/β-catenin signaling pathway[34]. Further studies are necessary in order to understand the role of STC2 played in cell proliferation, tumor growth and metastasis of EHCC.

Conclusions

In conclusion, BIRC7 and STC2 are involved in the tumorigenesis and progression of EHCC, and positive expression of BIRC7 and STC2 are associated with poor prognosis in patients with EHCC. All these findings suggest BIRC7 and STC2 is a potential tumor biomarker for EHCC diagnosis and prognosis.

Abbreviations

BIRC7: Baculoviral IAP repeat-containing 7; CA19-9: Carbohydrate antigen 19-9; CCA: Cholangiocarcinoma; EHCC: Extrahepatic CCA; EMT: Epithelial-mesenchymal; IHCC: Intrahepatic CCA; STC2: Stanniocalcin 2; TNM: Tumor node metastasis.

Declarations

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Non applicable.

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Availability of data and materials

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

Authors' contributions

LJQ carried out the studies, performed the statistical analysis and wrote the paper. YZL designed the study and revised the paper. LDQ and HSF collected specimens and experimental materials. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the ethical committee of Central South University. This study is a retrospective study, and the data collection and analysis were carried out without disclosing patients' identities.

Consent for publication

This study is exempted from an informed consent since it is a retrospective study and the data collection and analysis were carried out without disclosing patients' identities.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 2

Immunohistochemical staining of BIRC7, ×200A, Positive expression of BIRC7 in moderately-differentiated EHCC; B, Negative expression of BIRC7 in well differentiated EHCC; C, The positive expression of BIRC7 in pericancerous tissues of EHCC; D, The positive expression of BIRC7 in billiary tract adenoma.

Figure 3

Immunohistochemical staining of STC2×200A, Positive expression of STC2 in moderately differentiated EHCC; B, Negative expression of STC2 in well differentiated EHCC; C, The positive expression of STC2 in pericancerous tissues of EHCC; D, The positive expression of STC2 in billiary tract adenoma.

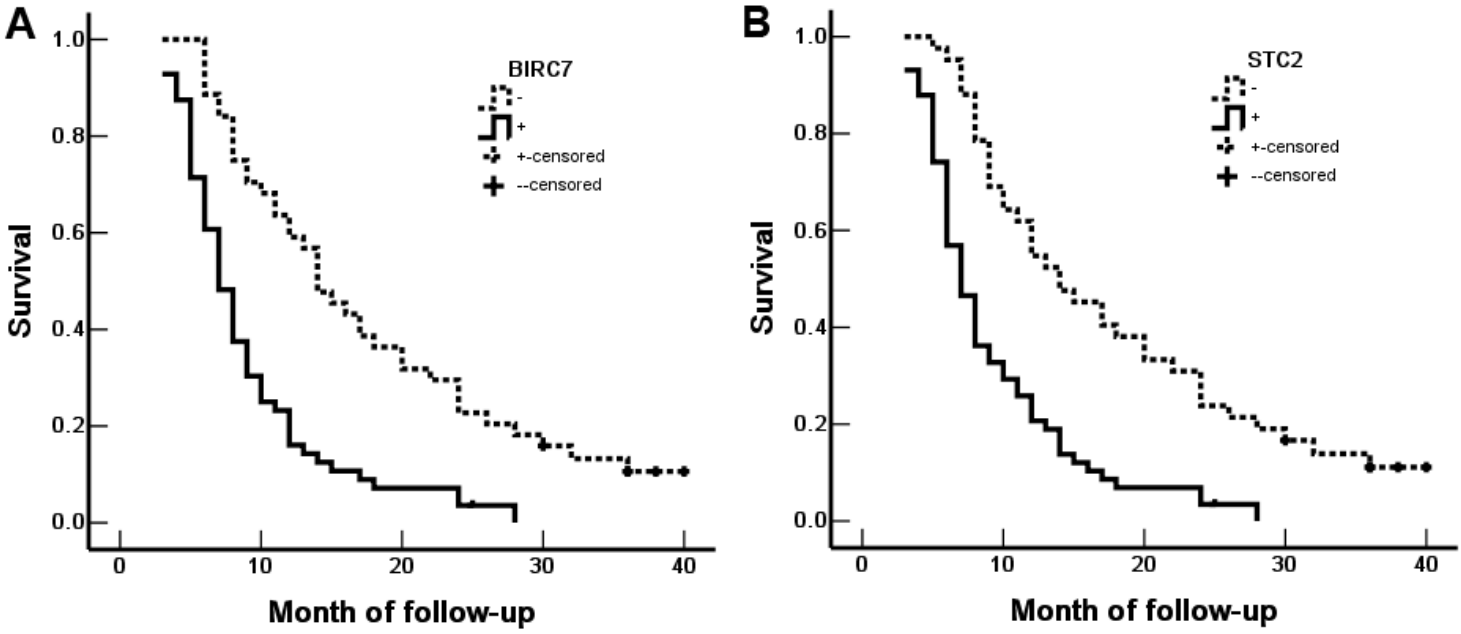

Figure 5

The expression of BIRC7 and STC2 and overall survival of EHCC patients. A, Kaplan-Meier curve for survival analysis of EHCC patients with BIRC7-positive and BIRC7-negative expression; B, Kaplan-Meier curve for survival analysis of EHCC patients with STC2-positive and STC2-negative expression.

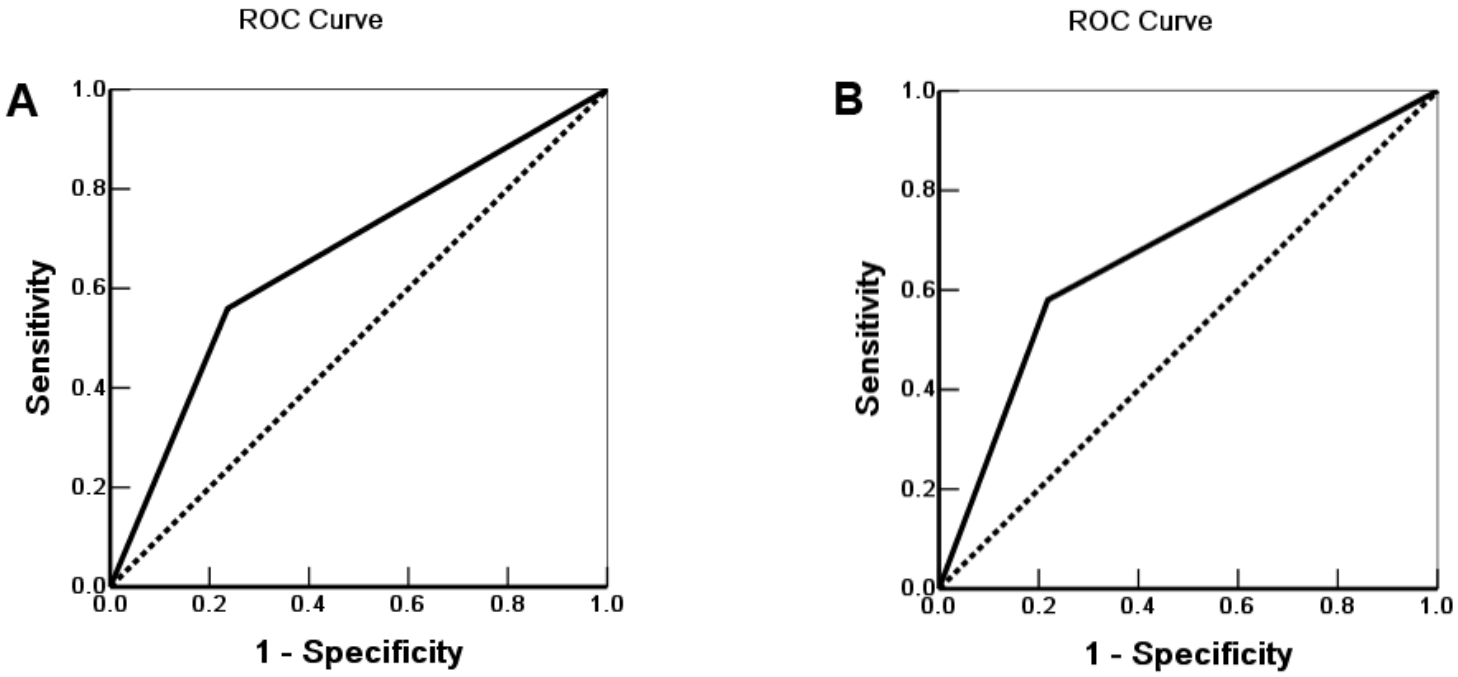

Figure 7

Multivariate analysis. ROC of Diagonal segments was produced by ties of BIRC7(A) and STC2 (B) in EHCC patients.