Surgical Oncology 29 (2019) 41–47

Contents lists available at ScienceDirect

Surgical Oncology

journal homepage: www.elsevier.com/locate/suronc

The expressions of HMGA2 and Thy1 in extrahepatic cholangiocarcinoma and their clinicopathological significances

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ARTICLE INFO

Keywords:
Extrahepatic cholangiocarcinoma
Biliary tract adenoma
HMGA2
Thy1
Immunohistochemistry

ABSTRACT

Aims: Extrahepatic cholangiocarcinoma is a malignant tumor and poor prognosis with intrinsic resistance to cytotoxic agents. The molecular mechanism associated with high malignancy and resistance to chemotherapy and radiotherapy has not been fully elucidated. This study aims to investigate the clinicopathological significances of HMGA2 and Thy1 expression in extrahepatic cholangiocarcinoma.

Methods: The expressions of HMGA2 and Thy1 in 100 extrahepatic cholangiocarcinoma, 30 peritumoral tissues, 10 adenoma and 15 normal biliary tract tissues were assayed using EnVision immunohistochemistry.

Results: The HMGA2 and Thy1 proteins were overexpression in extrahepatic cholangiocarcinoma compared to peritumoral tissues, adenoma, and normal biliary tract tissues ($P < 0.05$ or $P < 0.01$). Adenoma and pericancerous tissues with positive HMGA2 or/and Thy1 protein expression exhibited atypical hyperplasia. The positive correlation was found between the expression of HMGA2 and Thy1 in extrahepatic cholangiocarcinoma ($P < 0.01$). The positive rates of HMGA2 and Thy1 expression were significantly higher in cases with poor differentiation, lymph node metastasis, invasion, and TNM stage III or IV and no resection (biopsy only) ($P < 0.05$ or $P < 0.01$). Kaplan-Meier survival analysis showed that the survival of extrahepatic cholangiocarcinoma patients with positive HMGA2 and/or Thy1 expression is significantly shorter than patients with negative HMGA2 and/or Thy1 expression ($P = 0.000$). Cox multivariate analysis revealed that positive HMGA2 and/or Thy1 expressions were independently poor prognosis factors in extrahepatic cholangiocarcinoma patients. We calculated the AUC for HMGA2 (AUC = 0.610, 95%CI: 0.519–0.702), or Thy1 (AUC = 0.675, 95%CI: 0.588–0.762), respectively.

Conclusions: The present study indicated that positive HMGA2 and Thy1 expression are closely associated with the pathogenesis, clinical, pathological and biological behaviors, and poor prognosis in patients with extrahepatic cholangiocarcinoma.

1. Introduction

Cholangiocarcinoma, a tumor of biliary ducts, has a poor prognosis with only a 2% 5-year survival rate if the disease has spread outside the liver. Recently, the incidence of cholangiocarcinoma has significantly increased in the United States [1,2]. Several risk factors have been linked to the initiation, development and progression of this disease, such as age over 65, presence of biliary stones, chronic infection with liver flukes, hepatitis B and C viruses, inflammatory bowel disease, cirrhosis, and primary sclerosing cholangitis [1,2]. Cholangiocarcinoma is distinguished into intrahepatic cholangiocarcinoma (10%) and extrahepatic cholangiocarcinoma (90%). The extrahepatic cholangiocarcinoma is further characterized as perihilar (Klatskin) tumor (50%), tumor originating at the bifurcation of the common hepatic duct (10%), or tumor of the distal bile duct (40%) [3]. Obstructive jaundice is present in 90% of the patients with extrahepatic cholangiocarcinoma [4]. A pathological documentation of cholangiocarcinoma is not always feasible because of the difficulty in accessing the tumor site. Diagnosis usually relies on imaging modalities such as endoscopic retrograde cholangiopancreatography, which offers the option of tumor sampling for cytology, or magnetic resonance cholangiopancreatography [5]. In general, a cytologic diagnosis is achieved in only a minority of cases (15%–30%) [5]. Extrahepatic cholangiocarcinoma divided into four histologic grades: well differentiated, moderately

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https://doi.org/10.1016/j.suronc.2019.01.013
Received 16 January 2018; Received in revised form 10 January 2019; Accepted 27 January 2019

differentiated, poorly differentiated, and undifferentiated [6]. Tumor grade is an independent predictor of patient survival and disease recurrence [6]. In addition to grade, features such as lymphovascular invasion, lymph node metastasis, and macroscopic periductal tumor involvement are all of independent prognostic significance [7,8].

High mobility group protein A2 (HMGA2) is discovered in non-histone chromatin protein, which is closely related to tumorigenesis, invasion and metastasis of tumors. HMGA2 has high expression in epithelial or interstitial malignant tumors. Previous studies have revealed that HMGA2 positive expression is correlated with the metastasis of many malignant tumors, such as gallbladder cancer [9], oral squamous cell carcinoma [10,11], gastric cancer [12,13], endometrial serous carcinoma [14], renal carcinoma [15], esophageal cancer [16], pancreatic ductal adenocarcinoma [17], colorectal cancer [18], and so on. Besides, these previous studies showed that these patients with HMGA2 positive expression have poor prognosis. Thus, HMGA2 may play an important biological role in many malignant neoplasm. However, there is no report about HMGA2 expression in the benign and malignant lesions of the biliary tract.

Thymus cell antigen 1 (Thy1), also known as cluster of differentiation (CD) 90, is a 25–37 KD glycophosphatidylinositol-anchored protein that is expressed in numerous cell types, including T cells, neurons, endothelial cells, fibroblasts and numerous tumor cells. Functioning as an important regulator of cell-cell and cell-matrix interactions [19], Thy1 has also been proposed to be an important molecule in cancer. It is overexpressed during prostate cancer progression [20]. In hepatocellular carcinoma, increased Thy1 expression is associated with the presence of cancer [21]. It is noteworthy that Thy1 tends to be expressed in poorly differentiated hepatocellular carcinoma and is associated with poor prognosis [22,23]. Consistent with this, male patients with Thy1-positive breast cancer have significantly poorer survival than those with Thy1-negative expression [24]. In addition, Thy1 promotes migration and metastasis in melanoma [25]. Notably, it has been suggested that Thy1 has opposite functions in ovarian [26,27] and nasopharyngeal cancer [28], where it functions as a tumor suppressor. Nonetheless, the values of Thy1 in the context of extrahepatic cholangiocarcinoma remains undetermined.

The role of HMGA2 and Thy1 in extrahepatic cholangiocarcinoma remains to be clarified. Thus, we evaluated HMGA2 and Thy1 expression in surgically resected specimens, including extrahepatic cholangiocarcinoma, pericancerous tissues, adenoma and normal biliary tract, by using immunohistochemistry. The clinicopathological significance and prognostic values of HMGA2 and Thy1 expressions were evaluated.

2. Material and methods

2.1. Case selection

The present retrospective study was approved by the Ethics Committee for Human Research, Central South University, and was conducted according to the approved guidelines. One hundred extrahepatic cholangiocarcinoma, thirty peritumoral tissues, ten biliary tract adenoma, and fifteen normal biliary tract tissues were obtained at the Second and third Xiangya Hospitals, Central South University from January 2001 to December 2013. All specimens obtained from the patients were histologically confirmed by two pathologists. Tumors were restaged according to the 7th TNM Classification of Malignant Tumors and classified following the World Health Organization (WHO) tumor classification system. Tumor differentiated degrees were defined according to the WHO criteria (well differentiated, moderately differentiated and poorly differentiated).

Clinicopathological data for extrahepatic cholangiocarcinoma is summarized in Table 2. Among the 100 extrahepatic cholangiocarcinoma samples, 61 were from male patients and 39 were female (M/F = 1.56) and patient ages ranged from 35 to 80 (58.8 ± 10.2) years. Of the 100 extrahepatic cholangiocarcinoma, 31 were well-differentiated (31.0%), 34 were moderately differentiated (34.0%) and 35 were poorly differentiated (35.0%). Among the 100 patients with extrahepatic cholangiocarcinoma, invasion of region tissues and/or organs was found in 67 (67.0%); 38 (38.0%) had regional lymph node metastasis; and 31 (31.0%) had gallstones. According to TNM staging, 35 of the 100 patients with extrahepatic cholangiocarcinoma were stage I + II, 38 were stage III and 27 were stage IV. Surgery included radical resection for 54 (54.0%), Palliative resection for 36 (36.0%) and only biopsy for 10 (10.0%). Survival data for the 100 patients with extrahepatic cholangiocarcinoma was obtained through letters and/or telephone calls. The follow-up time was 30 months, and patients who survived longer than 30 months were included in the analysis as censored cases.

Thirty pericancerous tissues were collected from extrahepatic cholangiocarcinoma of radical resection, twenty male (66.6%) and patient ages ranged from 35 to 72 (48.5 ± 9.2) years. The pathological examination showed 12 normal tissues, 8 mild dysplasia, 6 moderately dysplasia and four severe dysplasia. Ten biliary tract adenoma tissues collected from operative resected specimens, six male (60.0%) and patient ages ranged from 33 to 70 (46.7 ± 10.2) years. The pathological examination showed 6 normal tissues, 2 mild dysplasia and 2 moderate tissues to severe dysplasia tissues. Fifteen normal biliary tract tissues were collected from contributors of liver transplantion and pathological examination being normal biliary tract tissues.

All tissues were treated with 4% formaldehyde for 24–48 h, and were then routinely embedded in paraffin.

2.2. Immunohistochemistry

Rabbit anti-human HMGA2 and Thy1 polyclonal antibody were purchased from Dako Corporation (Carpentaria, CA, USA). EnVisionTM Detection Kit was purchased from Dako Laboratories (CA, USA). Positive controls were provided with the EnVisionTM Detection Kit. EnVision immunohistochemistry of HMGA2 and Thy1 was performed by following the user manual. Briefly, 4 μm-thick sections were cut from paraffin-embedded tissues. The sections were deparaffinized and then incubated with 3% H_2O_2 in the dark 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 °C for 30 min. The sections were incubated with rabbit anti-human HMGA2 and Thy1 primary antibody (1:100 dilution) for 2 h 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. Five hundred cells from ten random fields were examined per section by 2 observers independently. An average of the percentages from two observers was used for final evaluation. Cases with positive cells ≥25% were considered positive whereas other cases were considered negative.

2.3. Statistical analysis

Data was analyzed using the SPSS 17.0 (statistical package for the Social Sciences, Version 17.0). The inter-relationship of HMGA2 and Thy1 with histological or clinical factors was analyzed using χ^2 test or Fisher's exact test. The overall survival of patients with extrahepatic cholangiocarcinoma was analyzed using Kaplan-Meier univariate survival analysis and log-rank tests. Multivariate analysis was performed with Cox proportional hazards model and the 95% confidence interval was calculated. A $P < 0.05$ was considered statistically significant.

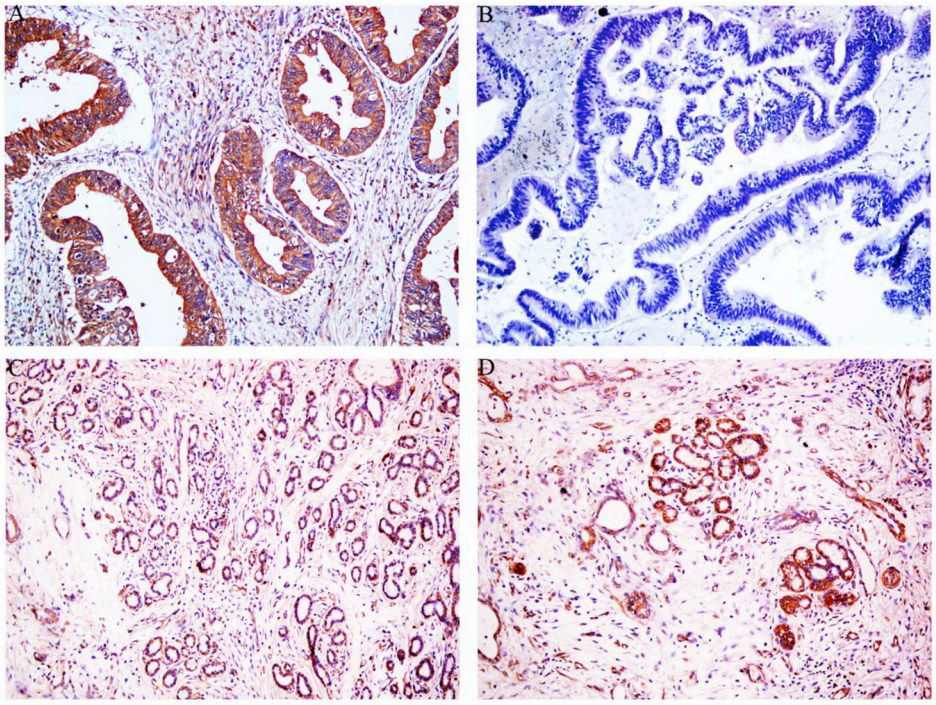

Fig. 1. Immunohistochemical staining of HMGA2, ×200. A. Positive expression of HMGA2, moderately-differentiated extrahepatic cholangiocarcinoma. B. Negative expression of HMGA2, well differentiated extrahepatic cholangiocarcinoma. C. The positive expression of HMGA2, pericancerous tissues. D. The positive expression of HMGA2, adenoma.

3. Results

3.1. HMGA2 and Thy1 protein expression in extrahepatic cholangiocarcinoma, peritumoral tissues, adenoma, and normal tissues

Immunohistochemical staining showed that HMGA2 and Thy1 proteins expressions were located in the cytoplasm (Figs. 1 and 2). In the one hundred extrahepatic cholangiocarcinoma, fifty-three and fifty-five were HMGA2 (53.0%) and Thy1 (55.0%) positive, respectively. In the thirty peritumoral tissues, nine and ten were HMGA2 (30.0%) and Thy1 (33.3%) positive, respectively. In ten adenoma, two and two were HMGA2 (20.0%) and Thy1 (20.0%) positive, respectively. In all fifteen normal tissues, HMGA2 and Thy1 expressions were negative. The positive rates of HMGA2 or Thy1 were significantly higher in extrahepatic cholangiocarcinoma than in peritumoral, adenoma and normal tissues ($P < 0.05$ or $P < 0.01$) (Table 1). Peritumoral tissues and adenoma with positive HMGA2 and/or Thy1 expression exhibited moderate to severe dysplasia (Table 1).

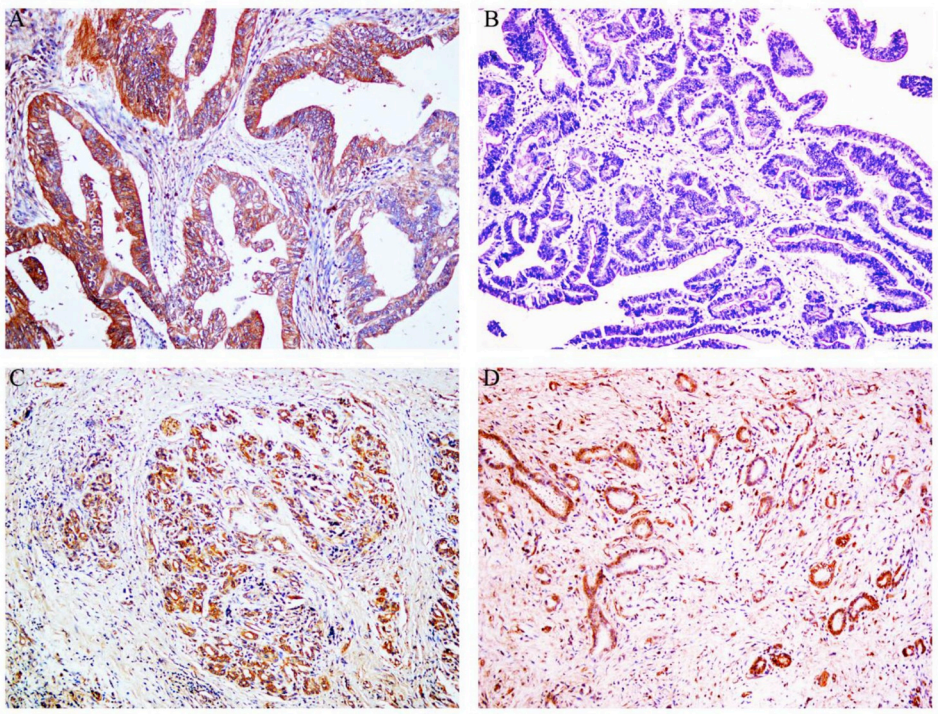

Fig. 2. Immunohistochemical staining of Thy1, ×200. A. Positive expression of Thy1, moderately differentiated extrahepatic cholangiocarcinoma. B. Negative expression of Thy1, well differentiated extrahepatic cholangiocarcinoma. C. The positive expression of Thy1, pericancerous tissues. D. The positive expression of Thy1, adenoma.

Table 1
Comparison of HMGA2 and Thy1 expression in normal, adenoma, peritumoral tissues and extrahepatic cholangiocarcinoma.

Tissue type	Number of Patients (N)	HMGA2 positive (%)	Thy1 positive (%)
extrahepatic cholangiocarcinoma	100	53 (53.0.0)	55 (55.0.0)
Peritumoral tissues	30	9 (30.0)*	10 (33.3)*
Adenoma	10	2 (20.0)*	2 (20.0)*
Normal tissues	15	0 (0.0)**	0 (0.0)**

Compared to extrahepatic cholangiocarcinoma: *$P < 0.05$; **$P < 0.01$.

3.2. HMGA2 and Thy1 protein expressions were associated with clinicopathological characteristics of extrahepatic cholangiocarcinoma

As shown in Table 2, positive rates of HMGA2 and Thy1 expression were significantly lower in cases with well differentiation, no metastasis in lymph node, no invasion to surrounding tissues and organs, TNM stage I + II and radical resection compared to cases with poor differentiation, lymph node metastasis, invasion, TNM stage III or IV and no resection (biopsy only) ($P < 0.05$ or $P < 0.01$). The positive rate of HMGA2 was significantly higher in the cases with biliary stone than in ones without biliary stone ($P < 0.05$). The expressions of HMGA2 and Thy1 exhibited no significant association with age, sex, tumor diameter and tumor sites ($P > 0.05$). Among the fifty-three cases with positive expression of HMGA2, thirty-nine cases exhibited Thy1 positive expression. Among forty-seven cases with negative expression of HMGA2, thirty-one cases showed negative Thy1 expression. The expression of HMGA2 was positively correlated with Thy1 in extrahepatic cholangiocarcinoma ($\chi^2 = 15.737$, $P = 0.000$).

3.3. HMGA2 and Thy1 protein expressions correlated with overall survival in patients with extrahepatic cholangiocarcinoma

Survival information of all patients was collected. Of the 100 patients with extrahepatic cholangiocarcinoma, fifty-nine patients died within twelve months, twenty-four patients died within twenty-four months, nine patients died within thirty months, and patients (eight cases) who survived longer than thirty months were included in the analysis as censored cases. Kaplan-Meier survival analysis revealed that the differentiation degree, lymph node metastasis, invasion, TNM stage and surgical procedure were significantly associated with the average overall survival time of patients with extrahepatic cholangiocarcinoma ($P < 0.05$ or $P < 0.01$) (Table 3). Average overall survival time of patients with positive expression of HMGA2 or Thy1 was significantly lower than those with negative HMGA2 or Thy1 expression ($P = 0.000$) (Fig. 3). Cox multivariate analysis showed that poor differentiation, lymph node metastasis, invasion, and a TNM stage of III or IV negatively correlated with overall survival and positively correlated with mortality. Positive HMGA2 or Thy1 expression negatively correlated with overall survival and positively correlated with mortality. Both HMGA2 and Thy1 expressions are independent prognostic factors (Table 4). Finally, we calculated the AUC for HMGA2 (AUC = 0.610, 95%CI: 0.519–0.702), or Thy1 (AUC = 0.675, 95%CI: 0.588–0.762), respectively (Fig. 4).

Table 2
Correlations of HMGA2 and Thy1 protein expression with the clinicopathological characteristics of extrahepatic cholangiocarcinoma.

CPC	Number of Patients (N)	HMGA2			Thy1		
		Pos No (%)	χ^2	*P* value	Pos No (%)	χ^2	*P* value
Age (year)							
≤45 years	17	11 (64.7)	1.127	0.288	9 (52.9)	0.035	0.851
> 45 years	83	42 (50.6)			46 (55.4)		
Sex							
Male	61	31 (50.8)	0.298	0.585	36 (59.0)	1.019	0.313
Female	39	22 (56.4)			19 (48.7)		
Differentiation							
Well	31	7 (22.6)	16.793	0.000	10 (32.3)	9.711	0.008
Moderately	34	22 (64.7)			21 (61.8)		
Poorly	35	24 (68.6)			24 (68.6)		
Tumor size							
≤3 cm	62	32 (51.6)	0.126	0.723	36 (58.1)	0.619	0.431
> 3 cm	38	21 (55.3)			19 (50.0)		
Tumor position							
Hilar site	27	17 (63.0)	1.475	0.478	12 (44.4)	0.703	0.704
Hepatic duct	4	2 (50.0)			1 (25.0)		
Distal duct	69	34 (49.3)			32 (46.4)		
Bile stone							
No	69	35 (50.7)	4.836	0.028	33 (47.8)	0.897	0.344
Yes	31	23 (74.2)			18 (58.1)		
Lymph node metastasis							
No	62	24 (38.7)	13.376	0.000	24 (38.7)	17.494	0.000
Yes	38	29 (76.3)			31 (81.6)		
Invasion							
No	33	10 (30.3)	10.186	0.001	11 (33.3)	9.342	0.002
Yes	67	43 (64.2)			44 (65.7)		
TNM stage							
I + II	35	9 (25.7)			10 (28.6)		
III	38	22 (57.9)	19.619	0.000	22 (57.9)	20.800	0.000
IV	27	22 (81.5)			23 (85.2)		
Surgery							
Radical	54	20 (37.0)			19 (35.2)		
Palliative	36	25 (69.4)	12.359	0.001	27 (75.0)	19.334	0.000
Biopsy	10	8 (80.0)			9 (90.0)		

Abbreviation: CPC, Clinicopathological characteristics; Pos No., Positive Number.

Table 3
Correlations of clinicopathological characteristics, HMGA2 and Thy1 expression with the mean survival in patients with extrahepatic cholangiocarcinoma.

Group	Number of Patients (N)	Mean survival (month)	χ^2	*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)		
Moderately	34	11.41 (3–30)	27.665	0.000
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				
I + II	35	18.57 (7–30)		
III	38	11.05 (3–30)	57.569	0.000
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)		
Palliative	36	7.58 (4–24)	48.388	0.000
Biopsy	10	6.90 (3–14)		
HMGA2				
−	47	18.43 (5–30)	37.157	0.000
+	53	8.21 (3–28)		
Thy1				
−	46	18.82 (6–30)	37.885	0.000
+	54	8.26 (3–30)		

Abbreviations: −, negative expression; +, positive expression.

4. Discussion

The expressions of HMGA2 and Thy1 have been associated with the progression and prognosis of a variety of tumors, but their expressions in extrahepatic cholangiocarcinoma have not been previously reported and their biological role in extrahepatic cholangiocarcinoma remains to be identified. Thus, we investigated HMGA2 and Thy1 protein expression in extrahepatic cholangiocarcinoma tumors, peritumoral tissues, adenoma, and normal biliary tract using immunohistochemistry in this study. We found that HMGA2 and Thy1 expression significantly increases in extrahepatic cholangiocarcinoma, compared with non-tumor tissues. In addition, Positive HMGA2 and Thy1 expressions are associated with poor differentiation, high TNM stages, invasion, metastasis, and poor prognosis of extrahepatic cholangiocarcinoma. These results suggested that HMGA2 and Thy1 may play an important biological role in extrahepatic cholangiocarcinoma.

As one of the high mobility protein family members, the HMGA2 protein was found in the late 1980s. This protein is encoded by the HMGA2 gene which is located on chromosome 12q14, 15, and has a molecular weight of approximately 12 KD. Many studies have revealed that HMGA2 has complex functions, and the current study focuses on its relationship with cancer. Previous studies have shown that HMGA2 gene expression in adult tissues was very low or had no expression, and was highly expressed in the early embryo and the epithelial or mesenchymal origin of malignant tumors, suggesting that the HMGA2 gene plays an important role in the growth of higher eukaryotes and in the proliferation and differentiation of malignant cells [29–31]. Being consistent with previous studies, we found that the positive rates of HMGA2 were significantly higher in extrahepatic cholangiocarcinoma than in peritumoral, adenoma and normal tissues. Recently, some studies have shown that HMGA2 expression was closely related to the progression, invasion, metastasis and prognosis of a number of malignant tumors, and tumors with high HMGA2 expression were highly malignant, and prone to invasive metastasis and poor prognosis [9–18]. Similarly, our data showed that HMGA positive expression is significantly increased in extrahepatic cholangiocarcinoma patients with poor differentiation, lymph node metastasis, invasion, a TNM stage of III or IV and no resection (biopsy only). This indicated that HMGA may play an key biological role in the occurrence and development of extrahepatic cholangiocarcinoma, which remains further identified.

Thy1 is a marker for several types of human stem cells, such as hematopoietic stem cells, hepatic stem/progenitor cells, and mesenchymal stem cells [32]. Thy1 is an important regulator of cell-cell and cell-matrix interactions, with significant roles in cellular adhesion and migration, nerve regeneration, and fibrosis [33]. Thy1 also plays important roles in oncogenesis and has been identified as a marker for cancer stem cells (CSCs) in various malignancies, such as liver cancer, esophageal cancer, gastric cancer, and glioma [22–25,34]. Thy1 + CSCs not only displayed tumorigenic capacity to initiate tumor and self-renewal, but also conferred an enhanced metastatic potential [35]. Besides the CSC properties of Thy1 + cells in these cancers, Thy1 expression has also been observed in stromal cells (e.g. mesenchymal stem

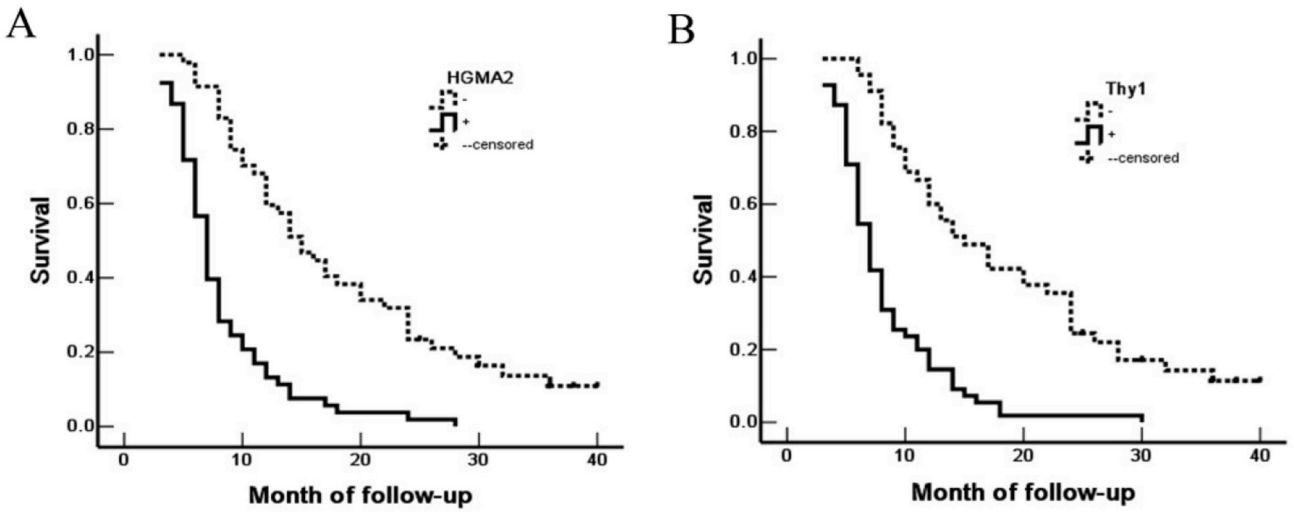

Fig. 3. HMGA2 and Thy1 expression and survival in patients with extrahepatic cholangiocarcinoma. A. Kaplan-Meier plots of overall survival in patients with HMGA2 -positive and -negative tumors. B. Kaplan-Meier plots of overall survival in patients with Thy1-positive and -negative tumors.

Table 4
Multivariate Cox regression analysis of survival rate in patients with pancreatic ductal adenocarcinoma and HMGA2 and Thy1 expression.

Groups	Factors	B	SE	wald	*P*	RR	95% CI	
							Lower	Upper
Differentiated degree	Well/moderately/poorly	0.469	0.155	9.156	0.002	1.598	1.180	2.166
Tumor size	≤3cm/ > 3 cm	0.473	0.235	4.051	0.044	1.605	1.012	2.544
Lymph node metastasis	No/Yes	0.823	0.311	7.003	0.008	2.277	1.238	4.189
Invasion	No/Yes	1.162	0.343	11.477	0.001	3.196	1.632	6.261
NM stage	I/II/III/IV	0.788	0.253	9.701	0.002	2.199	1.339	3.611
Surgery	Radical/Palliative/Biopsy	0.572	0.192	8.875	0.003	1.772	1.216	2.581
HMGA2	−/+	0.750	0.284	6.974	0.008	2.117	1.213	3.694
Thy1	−/+	0.759	0.253	6.747	0.007	2.169	1.219	3.687

Abbreviations: −, negative expression; +, positive expression; RR, relative risk, CI, confidence interval.

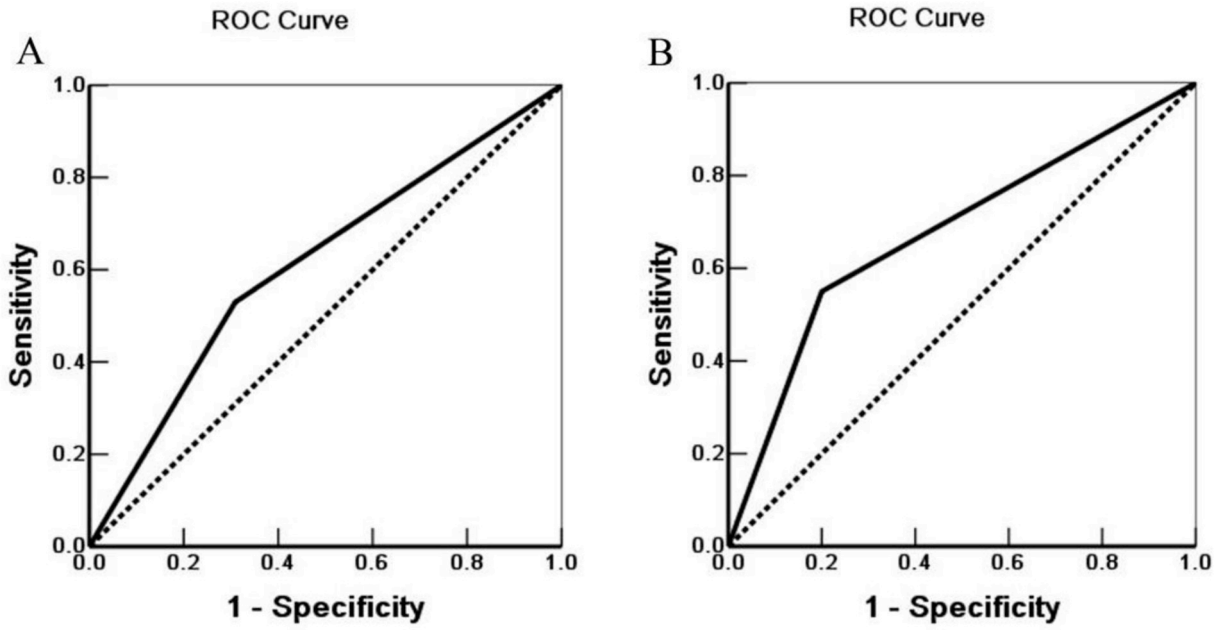

Fig. 4. ROC of Diagonal segments are produced by ties of HMGA2 (A)and Thy1 (B) in extrahepatic cholangiocarcinoma.

cells, cancer-associated fibroblasts, and endothelium) of various cancers, and plays an important role in disease progression [21,36,37]. Saalbach A et al. also have reported that Thy1 contributes to migration and metastasis of melanoma [25]. Likewise, our data showed that positive expression of Thy1 is closely associated with lymph node metastasis, invasion of extrahepatic cholangiocarcinoma. Previous studies have revealed that Thy1 positive expression is related to poor prognosis of hepatocellular carcinoma and breast cancer [22–24], which is consistent with our results that survival time of patients with Thy1 positive expression were shorter than those with Thy1 negative expression. Thus, Thy1 may play an important role in extrahepatic cholangiocarcinoma, which needs further study to identify its specific mechanism.

In the present study, we found that the percentage positive rates of HMGA2 and Thy1 expression were significantly lower in cases with well differentiation, no metastasis in lymph node, no invasion to surrounding tissues and organs, TNM I + II stage and radical resection compared to cases with poor differentiation, lymph node metastasis, invasion, and TNM III or IV stage and no resection (biopsy only) ($P < 0.05$ or $P < 0.01$). In biliary tract epithelia in pericancerous tissues and adenoma tissues with positive HMGA2 and Thy1 protein expression exhibited moderate to severe dysplasia. Kaplan-Meier survival analysis showed that extrahepatic cholangiocarcinoma patients with positive HMGA2 and Thy1 expression survived significantly shorter than patients with negative HMGA2 and Thy1 expression. Cox multivariate analysis suggested that positive HMGA2 and Thy1 expression are independent prognostic factors for poor prognosis in patients with extrahepatic cholangiocarcinoma. The AUC for HMGA2 and Thy1 showed that HMGA2 and Thy1 might play a role in carcinogenesis, progression and early finding or prevention of extrahepatic cholangiocarcinoma.

In conclusion, our study indicated that HMGA2 and Thy1 are involved in the tumorigenesis and progression of extrahepatic cholangiocarcinoma, and positive HMGA2 and Thy1 expressions were associated with poor prognosis in patients with extrahepatic cholangiocarcinoma.

Compliance with ethical standards

Conflict of interest

The authors declared no conflict of interest.

Ethical approval

The protocol of this study was approved by the Ethics Committee of Second Xiangya Hospital, Central South University. All procedures performed in studies involving human participants were in accordance

with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authorship

Zhulin Yang contributed to the conception and design of the study. Rushi Liu had participated in most of the research and article preparation. Shengfu Huang and Daiqiang Li had partly participated in acquisition of data and analysis and interpretation of data. Qiong Zou and Yuan had partly participated in analysis and interpretation of data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.suronc.2019.01.013.

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