RESEARCH COMMUNICATION

The Clinicopathological and Prognostic Impact of 14-3-3 Protein Isoforms Expression in Human Cholangiocarcinoma by Immunohistochemistry

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Abstract

The 14-3-3 proteins are highly conserved, ubiquitous molecules involved in a variety of biologic phenomena, such as cell cycle control, and apoptosis. However, their expression in cholangiocarcinoma has not been previously characterized. In this paper, immunohistochemistry using specific anti-14-3-3 monoclonal antibodies was performed on formalin-fixed;, paraffin embedded archival tissue from 86 patients of cholangiocarcinoma. We also examined the correlation between expression and survival rate and clinicopathologic factors such as tumor location, tumor size, pathologic differentiation, lymphatic permeation, lymph node metastasis, and tumor stage. Positive 14-3-3 proteins expression was observed for 6 isoforms (β, σ, γ, θ, δ, η) of these proteins in 86 patients of cholangiocarcinoma. β and σ isoform immunoreactivity was correlated with lymph node metastasis, tumor stage and patients' survival rate. In addition, δ isoform immunoreactivity showed trends with tumor location, tumor size, pathologic differentiation and tumor stage, while the θ isoform was correlated with pathologic differentiation. These results indicated that upregulated expression of some isoforms of 14-3-3 may be a common mechanism for evading apoptosis in cholangiocarcinoma, so that targeting 14-3-3 may be a novel promising strategy for the treatment of this tumor.

Keywords: 14-3-3 isoforms - immunohistochemistry - cholangiocarcinoma - malignant characteristics

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Introduction

Cholangiocarcinoma (CCA) is an adenocarcinoma arising from the epithelial tissue of the intra-hepatic (10%), hepatic hilar (25%) or extrahepatic (65%) bile ducts (Lim, 2003). It is accounting for approximately 3% of all gastrointestinal malignancies (Wijaya and Abdullah, 2011). Recent data show that the incidence of cholangiocarcinoma have been increasing in several areas worldwide (Shu et al., 2011), especially the incidence of intrahepatic CCA (Patel, 2001). Though several risk factors for developing cholangiocarcinoma have been identified, the pathogenesis of the disease remains poorly understood. Among gastrointestinal (GI) cancers, CCA is the most difficult to detect and diagnose (Mosconi et al., 2009), patients often have advanced stage disease at the time of diagnosis (Fava, 2010), leading to a poor clinical outcome (Tompkins et al., 1981; Tio et al., 1991; Miyakawa et al., 2009), the patient survival is usually measured in months, therefore it is of great importance to identify novel candidate markers and potential early indicators of this disease as well as molecules that may be potential therapeutic targets.

The 14-3-3 proteins are highly conserved eukaryotic proteins which are primarily found in high levels in neurones but which have also been shown to be expressed in a wide range of other cells and tissues. These proteins comprise seven distinct isoforms (β, σ, γ, θ, δ, ε, η), named from their reversephase high performance liquid chromatography elution profile.

14-3-3 proteins are involved in most of the cellular processes (Shankardas et al., 2008), including regulation of several metabolic pathways, redox regulation, transcription RNA processing, protein synthesis, folding and degradation, cell cycle, apoptosis, cytoskeletal organization and cellular trafficking by binding to phosphorylated sites in diverse target proteins (over 300 phosphoproteins identified). Considering the number of binding partners, it is not surprising that 14-3-3 proteins play crucial roles in regulating multiple cellular processes. In addition to their important roles in many normal physiology processes, 14-3-3 proteins have attracted much recent interest in the etiopathogenesis of human cancers owing to their involvement in the prevention of apoptosis.

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It has been reported that 14-3-3 proteins are overexpressed in some human diseases and have relations with them, particularly in cancers, including stomach cancers, breast cancer, and oral squamous cell carcinomas. However, its protein expression and clinicopathologic correlation in cholangiocarcinoma have not been reported.

To study the possible role of 14-3-3 proteins in cholangiocarcinoma, we were interested to examine the expression of these proteins on a total of 86 cases of surgically resected cholangiocarcinoma by immunohistochemistry with a specific anti-14-3-3 antibody. In addition, we investigate the correlation between 14-3-3 proteins expression and the patients' clinical outcome.

Materials and Methods

Cases and tissues

Eighty-six surgically resected samples were selected from patients pathologically diagnosed with cholangiocarcinoma with no preceding therapy at the Department of General Surgery, Peking Union Medical College Hospital (PUMCH), Beijing, China, from January 2000 to September 2010. This project was approved by the ethical committees of the hospital and informed consent was obtained from the participating patients. Cholangiocarcinoma was classified as intrahepatic or extrahepatic carcinoma depending on radiologic findings by computed tomography, magnetic resonance imaging, endoscopic retrograde cholangiopancreatography, or magnetic resonance cholangiopancreatography. Tumors arising in the intrahepatic bile ducts were classified as intrahepatic carcinoma (n=8) and in the extrahepatic bile ducts were classified as extrahepatic carcinoma (n=76), including Klatskin's tumors that occur at the bifurcation of the left and right hepatic ducts. Tumors were histologically divided into 3 groups (28 well differentiated, 44 moderately differentiated, and 14 poorly differentiated), according to their degree of papillary or tubular formation. If more than 1 type was found, the predominant type was recorded. The following histologic features were also examined: tumor location, tumor size, pathologic differentiation, lymphatic permeation, lymph node metastasis, and tumor stage. The tumor histologic stage was defined as stage I (n = 4), stage II (n = 28), stage III (n = 27), stage IVA (n=24) or stage IVB (n = 3) by histologic examination based on the pTNM classification proposed by the International Union Against Cancer.

Tissues were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin for histologic examination. A paraffin block used for routine pathologic diagnosis and containing representative tumor histology was selected in each case, and 3 μm-thick paraffin sections were prepared from this block for immunohistochemical analysis. Frozen tissues were obtained immediately after surgical resection and stored frozen at -80 °C.

Tissue preparation

The paraffin-embedded tissue sections of cholangiocarcinoma were dewaxed and rehydrated. After incubation with 3% hydrogen peroxide in absolute methanol to block endogenous peroxidase activity for 10 minutes, antigen retrieval was carried out according to the method shown in Table 1. The tissue sections were incubated at 37 °C for one hour in a wet container with the specific antibodies against seven 14-3-3 isoforms (Santa Cruz, CA), diluted in primary antibody diluting buffer (Dako). Then, the slides were incubated with a secondary antibody (Dako) for 50 minutes at room temperature. The tissue sections were visualized with 3, 3-diaminobenzidine (DAB) and counterstained with Mayer's hematoxylin. Finally, the slides were viewed with an Axiophot microscope (CarlZeiss, Germany) coupled with a ProgRes charge-coupled device (CCD) camera (Jenoptik, Germany).

Semiquantitative analysis of immunoreactivity

The intensity of IHC staining in the tumor cells was scored independently by two pathologists who were blinded to the clinicopathological data according to the semi-quantitative IRS (immunoreactive score) scale. The average value from the two referees was used as the final score. The 14-3-3 staining intensity (14-3-3-SI), percentage of 14-3-3-positive tumor cells (14-3-3-PP), and a resulting 14-3-3 immunoreactivity score (14-3-3- IRS) were assessed as a modification of the technique described previously for estrogen and progesterone receptors. In short, this immunoreactivity score (14-3-3-IRS: negative 0; weak 1–3; moderate 4–6; strong 8–12) was determined by multiplication of the values for 14-3-3 staining intensity (14-3-3-SI: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) and the values for percentage of 14-3-3-positive tumor cells (14-3-3-PP: score 1, 0-10%; score 2, 11-25%; score 3, 26-50%; score 4, > 51%).

Statistical analysis

All statistical analyses were done using SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL). To trace correlations between 14-3-3 proteins expression and several clinicopathologic parameters, data were cross-tabulated and Fisher exact test was done. The correlation of 14-3-3 proteins staining with patient survival was evaluated using life tables constructed from survival data with Kaplan-Meier plots. Comparisons of the different groups were done with the log-rank test. The end point in the present study was overall survival ranging from date of surgery until date of death or, if no information was documented, until date of last follow up information (=censored). Results were considered significant when $P < 0.05$.

Results

Immunohistochemical staining for 14-3-3 proteins in cholangiocarcinoma

We first did seven 14-3-3 isoforms immunohistochemistry staining on cancer tissues obtained from a cohort of 86 cholangiocarcinoma patients with different clinical stages and histologic types and grades. Immunohistochemical results are summarized in Figure 1.

Immunoreactivity for 14-3-3 proteins in cancerous

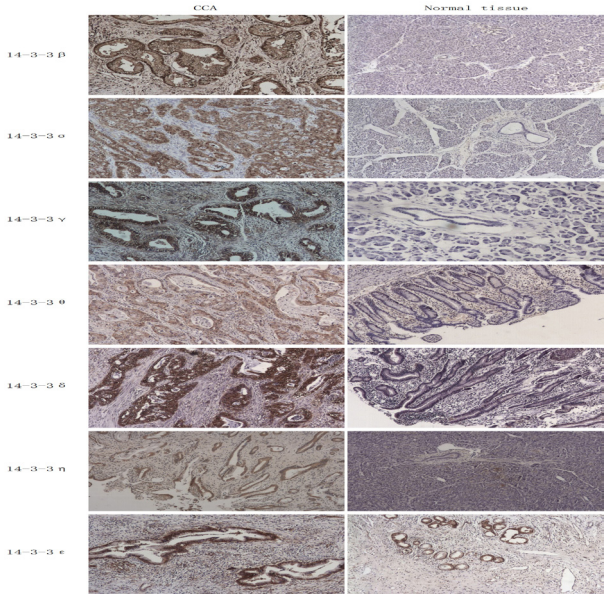

Figure 1. Immunohistochemical Staining of 14-3-3 Proteins in Cancerous Tissue and Adjacent Normal Bile Ducts. Increased expression of 14-3-3 β, σ, γ, θ, δ and η isoforms was observed in cholangiocarcinoma

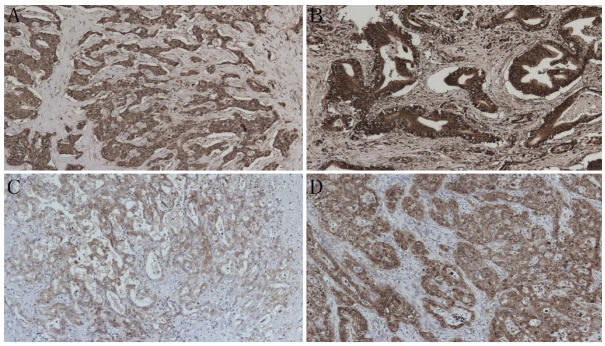

Figure 2. The Correlation Between 14-3-3 β, σ Isoforms Expression and Lymph Node Metastasis. (A) and (C): The expression of 14-3-3 β and σ in cholangiocarcinoma with negative lymph node metastasis. (B) and (D): The expression of 14-3-3 β and σ in cholangiocarcinoma with positive lymph node metastasis

tissue was observed mainly in cytoplasm and/or nuclear. The overexpression of 14-3-3 proteins in cholangiocarcinoma tissue were observed in isoforms of β, σ, γ, θ, δ and η, their total positive staining rates were 95.3%, 59.3%, 68.6%, 68.6%, 85.6% and 50.6% respectly. There was a significant difference of 14-3-3 proteins immunoreactivity between cholangiocarcinoma tissue and adjacent normal bile ducts. The positive immunohistochemistry staining of 14-3-3 ε was observed in 66 of 86 adenocarcinoma (76.7%) and 62 of 86 normal bile ducts (72.1.6%) (P = 0.06), suggesting that there was no significant difference of 14-3-3 ε expression between cholangiocarcinoma tissue and adjacent normal bile ducts.

Correlation between 14-3-3 proteins expression and clinicopathologic parameters

The correlative analysis between 14-3-3 proteins expression and the clinicopathologic characteristics of patients with cholangiocarcinoma is summarized in Table 2.

High expression of 14-3-3 β and σ were significantly correlated with lymph node metastasis and tumor

Table 1. Details of the Primary Monoclonal Used in the Present Study

Antigen	Clone	Dilution	Antigen retrieval
14-3-3β	sc-25276	1:500	B
14-3-3σ	sc-100638	1:50	A
14-3-3γ	sc-731	1:50	B
14-3-3θ	sc-732	1:50	A
14-3-3δ	sc-1019	1:200	B
14-3-3ε	sc-23957	1:100	B
14-3-3η	sc-17287	1:100	A

NOTE: A, Heat in an autoclave for 5 minutes in citric acid buffer (pH 6.0); B, Microwaved in citrate buffer (pH 6.0) at 99°C

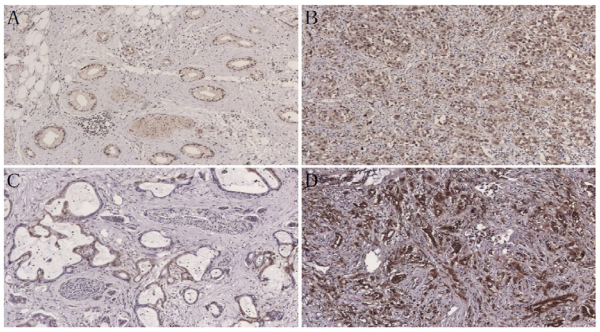

Figure 3. The correlation between 14-3-3θ,δisoforms expression and pathologic differentiation. (A) and (C): The expression of 14-3-3θandδin well differentiation cholangiocarcinom. (B) and (D): The expression of 14-3-3θandδin poor differentiation cholangiocarcinoma

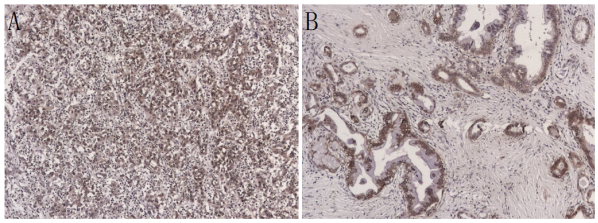

Figure 4. The Correlation Between 14-3-3 δ Isoform Expression and Tumor Location. (A): The expression of 14-3-3 δ in intrahepatic carcinoma. (B): The expression of 14-3-3 δ in extrahepatic carcinoma

stage of those with cholangiocarcinoma. In 44 cases of cholangiocarcinoma with positive lymph node metastasis, the total positive staining rates of 14-3-3 β and σ were 100% and 81.8%. In 42 cases without lymph node metastasis, the total positive staining rates of these two isoforms were 88.1% and 35.7%. These results indicated that the patients with positive immunohistochemistry staining of 14-3-3 β and σ were presented a higher frequency of lymph node and distant metastasis (P = 0.024, 0.000, Figure 2), and a more advanced clinical stage (P = 0.017, 0.000).

High expression of 14-3-3 θ and δ were significantly correlated with pathologic differentiation. Increased expression of 14-3-3 θ protein was observed in 8 of 28 well differentiation (28.6%), 31 of 58 moderate and poor differentiation (54.4%) (P = 0.038). Increased expression of 14-3-3 δ protein was observed in 15 of 28 well differentiation (53.6%), 45 of 58 moderate and poor differentiation (77.6%) (P = 0.043). The results showed significant increase in 14-3-3 θ and δ expression was

Table 2 (A). Relationship Between 14-3-3 Proteins Expression and Clinicopathologic Variables

c	14-3-3β		P	14-3-3σ		P	14-3-3γ		P
	+(n=82)	-(n=4)		+(n=51)	-(n=35)		+(n=59)	-(n=27)	
Sex(M/F)	47/35	3/1	NS	31/20	19/16	NS	35/24	15/12	NS
Age (y)[a]	59.3±9.0	51.7±17.9	NS	58.1±9.7	60.1±9.3	NS	59.7±9.9	57.1±8.7	NS
Location			NS			NS			NS
ICC	8/8	0/8		5/8	3/8		6/8	2/8	
ECC	74/78	4/78		46/78	32/78		53/78	25/78	
Size[a]	3.5±2.0	1.8±1.2	NS	3.4±1.9	3.3±2.1	NS	3.3±1.9	3.6±2.3	NS
Differentiation			NS			NS			NS
Well	26/28	2/28		9/28	19/28		12/28	16/28	
Mod/Poor	56/58	2/58		23/58	35/58		34/58	24/58	
Lymph node metastasis			0.045			0			NS
YES	45/45	0/45		36/45	9/45		34/45	11/45	
NO	37/41	4/41		14/41	27/41		32/41	9/41	
TNM stage			0.017			0			NS
I+II	28/32	4/32		10/32	22/32		22/32	10/32	

Table 2 (B). Relationship Between 14-3-3 Proteins Expression and Clinicopathologic Variables

c	14-3-3θ		P	14-3-3δ		P	14-3-3ε		P	14-3-3η		P
	+(n=59)	-(n=27)		+(n=71)	-(n=15)		+(n=66)	-(n=20)		+(n=40)	-(n=39)	
Sex(M/F)	32/27	18/9	NS	43/28	7/8	NS	38/28	12/8	NS	25/15	21/18	NS
Age (y)[a]	59.3±10.1	58.0±8.3	NS	59.4±8.9	56.6±12.3	NS	59.3±9.5	57.5±9.8	NS	59.0±9.8	58.8±9.4	NS
Location			NS			0.047			NS		S	N
ICC	6/8	2/8		8/8	0/8		59/78	19/78		35/71	36/71	
ECC	53/78	25/78		63/78	15/78		59/78	19/78		35/71	36/71	
Size[a]	3.6±2.3	3.0±1.0	NS	3.5±2.1	2.5±0.9	NS	3.5±2.1	2.7±1.1	NS	3.6±2.0	3.3±2.1	NS
Differentiation			0.038			0.043			NS			NS
Well	8/28	20/28		15/28	13/28		14/28	14/28		8/24	16/24	
Mod/Poor	31/58	27/58		45/58	13/58		37/58	21/58		7/55	48/55	
Lymph node metastasis			NS			NS			NS			NS
YES	35/45	10/45		39/45	6/45		34/45	11/45		22/42	20/42	
NO	24/41	17/41		32/41	9/41		32/41	9/41		18/37	19/37	
TNM stage			NS			NS			NS			NS
I+II	18/32	14/32		24/32	8/32		25/32	7/32		13/27	14/27	

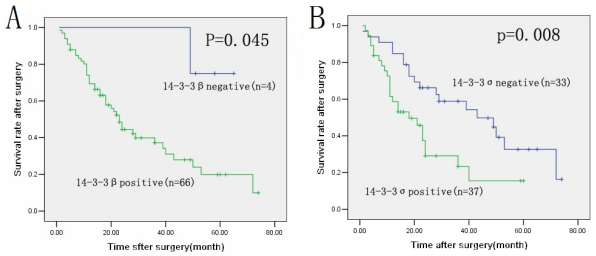

Figure 5. The Correlation Between 14-3-3 β, σ Isoforms Expression and Patients Survival Rate. (A): Survival curves of patients in the 14-3-3 β positive and 14-3-3 β negative groups. (B): Survival curves of patients in the 14-3-3σpositive and 14-3-3σnegative groups. Patients in the negative group had significantly worse survival than those in the positive group

observed in different histological grades, Figure 3. In addition, high expression of 14-3-3 δ was significantly correlated with tumor location and tumor stage. In 8 cases of intrahepatic carcinoma, all the tumoral tissue were stained positively, and in 78 extrahepatic carcinoma cases, the percentage of positive immunohistochemistry staining of 14-3-3 δ was 80.8%(63/78), so there was a increase expression of 14-3-3 δ in intrahepatic carcinoma than extrahepatic carcinoma, Figure 4.

None of the examined clinicopathologic parameters (sex, age, tumor location, tumor size, pathologic differentiation, lymphatic permeation, lymph node metastasis, and tumor stage) were significantly correlated to 14-3-3 γ and η expression.

Table 3. Multivariate Analysis by the Cox Proportional Hazards Model

Variables	Hazard ratio	95% confidence interval	P
Advanced tumor stage	2.014	1.296-3.539	0.001
Lymph node metastases	1.600	1.064-2.595	0.045
Over expression of 14-3-3σ	1.858	1.390-3.101	0.027

Survival analysis

Seventy of 86 (81.4%) patients had follow-up clinical outcome. Survival analysis according to the Kaplan-Meier method revealed that the survival of patients in the 14-3-3 β and σ positive group was significantly poorer than that of patients in the negative group. The 1-, 3-, and 5-year survival rates were 100%, 100%, and 75% in the 14-3-3 β negative group compared with 72.7%, 22.7%, and 6.1% in the 14-3-3 β positive. The 1-, 3-, and 5-year survival rates were 90.9%, 42.4%, and 12.1% in the 14-3-3 σ negative group compared with 59.5%, 13.5%, and 2.7% in the 14-3-3 σ positive. The data suggest that the overall survival probability was significantly different between the two groups (P = 0.045, P = 0.008, respectively, log-rank test; Figure 5).

By Univariate survival analysis, the following were poor prognostic factors for cholangiocarcinoma: lymph

node metastases, poor histological differentiation, advanced tumor stage (III+IV), overexpression of 14-3-3 β and σ. Furthermore, all 5 of these variables were entered into a stepwise multivariate proportional hazard model, which revealed that the loss of 14-3-3σexpression is an independent prognostic factor (P = 0.027), as are advanced tumor stage (P =0.001) and lymph node metastases (P = 0.045) (Table 3).

Discussion

The 14-3-3 proteins have emerged as critical regulators of diverse cellular responses in eukaryotic organisms (Zang et al., 2010), they can form homodimers or heterodimers that allow them to function as an adapter, linker, scaffold or coordinator in assembling signaling complexes (Wilker et al., 2004). Because of its importance in the regulation of key signal transduction pathways, deregulation of 14-3-3 has been associated with pathological consequences (Porter et al., 2006; Kilani et al., 2007). Recent studies have suggested that 14-3-3 proteins are potential oncogenes (Tzivion et al., 2006). To our knowledge, it is the first time to evaluate the expression of seven different 14-3-3 isoforms using immunohistochemistry method and their possible roles in conjunction with clinical outcome of the patients in cholangiocarcinoma, it is important to clarify the relationship between 14-3-3 family proteins with the carcinogenesis of cholangiocarcinoma. In tumoral tissue, six isoforms, 14-3-3 β, σ, γ, θ, δ and η, were present in abundance. These data indicate that these isoforms may be involved in bile duct tumorigenesis. Because 14-3-3 proteins participate in important cellular events including cell cycle checkpoint, signal transduction, apoptosis and cell division, it may be that these processes are dysregulated in these tumors. It has been found recently that human cruciform binding protein belongs to theβ, ε,σ,δandγisoforms of the 14-3-3 family (Todd et al., 1998). These five 14-3-3 proteins form a complex that binds to cruciform DNA to stimulate DNA replication. Given the transformed cells need more DNA replication to support the cells proliferation, the elevated number and quantity of isoforms that we found in cholangiocarcinoma may reflect the cancer cell growth requirements.

Similarly as previous reports, we demonstrated that 14-3-3 β was overexpressed frequently in cholangiocarcinoma, suggesting 14-3-3 β might have oncogenic potential. 14-3-3βcan bind integrin (Zhai et al., 2001), testicular protein kinase 1 (Toshima et al., 2001) and Wee1 (Wang et al., 2000) to regulate cell spreading and G2-M transition. Notably, overexpression of 14-3-3 β has a role in the proliferation and oncogenic transformation of NIH 3T3 cells. Sugiyama reported that reduction of 14-3-3 β in rat hepatoma K2 cells diminishes their growth ability and tumorigenicity in nude mice (Sugiyama et al., 2003). Importantly, 14-3-3 β mRNA was overexpressed in various murine tumor cell lines and reduction of 14-3-3 β could suppress tumor cell growth in vitro and in vivo.

14-3-3 σ was identified as an epithelial-specific marker (Guweidhi et al., 2004), functionally, it is associated with control of the G2/M checkpoint in the cell cycle. The expression of 14-3-3 σ is induced downstream of the activation of p53 and results in a G2/M arrest. Loss of 14-3-3 σ expression has been reported in primary gastric (Suzuki et al., 2000), hepatocellular (Iwata et al., 2000), and breast adenocarcinoma (Jeanteur, 2000). In contrast, 14-3-3 σ expression is up-regulated in head and neck squamous cell carcinoma and in chemoresistant pancreatic adenocarcinoma cells (Sinha et al., 1999). In our study, 14-3-3 σ was overexpressed in cholangiocarcinoma. The biological significance of 14-3-3 σ expression in cholangiocarcinoma may be its anti-apoptotic effect like in pancreatic carcinogenesis, achieved by inhibiting the proapoptotic proteins BAD and BAX (Zha et al., 1996; Samuel et al., 2001; Nomura et al., 2003). Therefore, due to diversity of 14-3-3 σ functions, its role in cancerogenesis might be restricted to the particular type of tumor.

Furthermore, we found that 14-3-3 β and σoverexpression were mainly found in more advanced tumor stages and predicts poor overall survival in this patient group indicating that 14-3-3 β and σ may be associated with an aggressive biological characteristics in cholangiocarcinoma progression, which play an important role in prognosis and/or recurrence, although it could be a relatively early event during their carcinogenesis.

14-3-3 δ is one of the major transforming growth factor-b-induced proteins, which can promote epithelial-mesenchymal transition of epithelial cells in cancer cell transformation (Keshamouni et al., 2006). In the present study, the upregulation of 14-3-3 δ was observed in cholangiocarcinoma. This result suggests that the δ isoform of 14-3-3 is dysregulated in tumors. 14-3-3 δ was proposed to be involved in apoptosis through multiple interactions with proteins of the core mitochondrial machinery, proapoptotic transcription factors, and their upstream signaling pathways. Our study showed that 14-3-3 δ expression is positively correlated with pathologic differentiation, and tumor stage. Thus, increased expression of 14-3-3 δ contributes to cholangiocarcinoma development and progression, and the detection of the 14-3-3 δ aberrations might be a useful biomarker to identify poor prognoses in patients with cholangiocarcinoma. More importantly, our data first indicate that 14-3-3 δ expression is positively correlated with tumor location, there were more 14-3-3 δ expression in intrahepatic carcinoma than extrahepatic carcinoma.

In our study, we also found the overexpression of 14-3-3 θ, γ, η in cholangiocarcinoma compared with adjacent normal bile ducts. These data indicate that the increased expression of the three 14-3-3 isoforms may contribute to bile duct tumorigenesis. Previous study has proved that a region from α-helix 7 to the C terminus of 14-3-3 θ was crucial for the interaction with Bax, the caspasedependent dissociation of Bax from 14-3-3 θ acts as an initial trigger for apoptotic mitochondrial changes. The overexpression of 14-3-3 θ can lead to cell adhesion to tenascin-C and an enhanced growth rate of tumor cells (Martin et al., 2003), it was overexpressed in human astrocytomas and lung adenocarcinoma and squamous cell carcinoma. Furthermore, we also found the expression of 14-3-3 θ is improved with the increasing of pathological grades of cholangiocarcinoma, like 14-3-3 δ. One reasonable explanation for this phenomenon may be that comparing

to the lower-grade cholangiocarcinoma, the higher-grade cholangiocarcinoma have relatively insufficient blood supply and are more susceptive to apoptosis-inducing signals theoretically, in order to be resistant to these pro-apoptotic signals and to gain survival advantages. One possible option for high grades astrocytomas cells is to produce more 14-3-3 θ and δ, and which in turn exert their anti-apoptotic functions.

The overexpression of 14-3-3 ε contributes to remodeling of extracellular matrix in skin through increasing MMP-2 gene expression via p38 MAPK signaling (Lee et al., 2009). However, we noticed that 14-3-3 ε protein had similar expression levels between cholangiocarcinoma tissue and adjacent normal bile ducts in our studies, which was different from its higher expression in renal carcinoma (Liang et al., 2009). This difference was mainly due to the following reasons. The tissue-specific interactions with 14-3-3 ε are different, in combination with the potential heterodimerization, which were mainly responsible for its differed expression level in specific tumors. In addition, the methodology to compare 14-3-3 ε expression was different in other the group's report and our studies.

In summary, this study documents the first analysis of 14-3-3 proteins expression in cholangiocarcinoma. The results show that 14-3-3 is expressed in majority of cases, although with different intensity and distribution. The results also suggest that up-regulated expression of 14-3-3 β, σ, γ, θ, δ, η isoforms may be related to occurrence or progression of cholangiocarcinoma, and target 14-3-3 may be a novel promising strategy for the treatment of cholangiocarcinoma and targets for therapy.

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