

Intrahepatic sarcomatoid cholangiocarcinoma of round cell variant: a case report and immunohistochemical studies

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Received: 3 March 2006 / Accepted: 4 August 2006 / Published online: 11 October 2006
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Abstract Sarcomatoid transformation was observed in 4.5% of autopsied cases of intrahepatic cholangiocarcinoma. Here, we report a case of intrahepatic sarcomatoid cholangiocarcinoma with round cell feature, extremely rare variant. An 87-year-old man was incidentally found to have a tumor in the left lobe of the liver by abdominal computed tomography scans. The patient was clinically diagnosed to have intrahepatic cholangiocarcinoma and received palliative care without specific treatment. He died of hepatic insufficiency 3 months after the diagnosis. The autopsied liver specimens showed a reddish gray tumor of 4.0×2.8 cm in size. Histologically, the tumor was centrally hemorrhagic and necrotic and was composed of tubular adenocarcinoma and a round cell component, which has an eccentrically located nucleus and eosinophilic cytoplasm without mucin production. Immunohistochemically, the adenocarcinoma cells expressed cytokeratin 19 and β -catenin in their cytoplasm, with E-cadherin and CD44s at the plasma membrane. In the round cells, cytokeratin 19 and vimentin was detected in their cytoplasm and CD44s at the plasma membrane. E-cadherin immunoreactivity was weakly present in their cytoplasm and β -catenin was negative. Loss or reduction of the E-cadherin and β -catenin expressions and overexpression of CD44s in the round cells are suggested to

be contributed to the high propensity for lymphatic permeation and poor prognosis.

Keywords Intrahepatic sarcomatoid cholangiocarcinoma · Round cell variant · E-cadherin · β -catenin · CD44s

Introduction

Sarcomatoid transformation has been observed in carcinomas in various organs. In the liver, sarcomatoid transformation was present in 3.9% (14 of 355) to 9.4% (55 of 579) of hepatocellular carcinoma (HCC) autopsy cases [10, 11] and 4.5% (7 of 155) of cholangiocarcinoma cases [17]. The sarcomatoid components usually show spindle-shaped cells, pleomorphic cells or multinucleated giant cells in previously reported cases of intrahepatic sarcomatoid cholangiocarcinoma (ISC) [5, 6, 8, 9, 14, 16, 17, 20, 21] and combined hepatocellular and cholangiocellular carcinoma with sarcomatoid changes [5, 15, 18]. However, intrahepatic cholangiocarcinoma (ICC) with round cell feature is extremely rare [16].

Cell adhesion molecules are considered to be involved in tumor invasion and metastasis. Cadherins are Ca^{2+} -dependent cell–cell adhesion molecules that interact with catenins, which are submembranous cytoplasmic proteins termed α -, β -, and γ -catenin [19]. Reduced expressions of E-cadherin, which are constantly expressed in epithelial cells, and catenins have been reported in many cancers. In cholangiocarcinoma, down-regulated expressions of E-cadherin and β -catenin have been reported to be significantly correlated with high grade tumors but not with vascular invasion and metastasis [2].

CD44 standard (CD44s) is a cell adhesion molecule, specifically a transmembrane glycoprotein that functions as

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a hyaluronate receptor, which may modulate cell–cell or cell–matrix interactions [23]. Aberrant expressions of CD44 have been observed in a variety of carcinomas [23]. In the bile duct, normal epithelial cells never express CD44s, even weakly or focally, in a nonspecific manner [2, 23, 24]. There have been a few reports of aberrant CD44s expressions in bile duct carcinoma cells, although no relationships of the CD44s immunoreactivity with the tumor grade, vascular invasion, and metastasis were noted [2, 24].

To date, no studies have investigated the above-described molecules in cholangiocarcinoma with sarcomatoid transformation. Here, we described a case of ISC of round cell variant and examined the differences between the immunohistochemical profiles of the tubular adenocarcinoma and the round cells, with particular focus on E-cadherin, β -catenin, and CD44s.

Clinical history

An 87-year-old man was incidentally found to have elevated ductal enzyme levels. His family history was unremarkable. He had never suffered from liver disease. Laboratory data revealed a moderately elevated serum level of carcinoembryonic antigen (16.2 ng/ml) and a highly elevated level of CA19-9 (2,894 U/ml). The serum level of alpha-fetoprotein was within the normal range. Hepatitis B surface antigen and anti-hepatitis C virus antibody in the serum were negative. Abdominal computed tomography

(CT) scans showed a tumor in the left lobe of the liver. The patient was clinically diagnosed to have ICC. He received palliative care without surgery or chemotherapy and died of hepatic insufficiency and bronchopneumonia 3 months after the diagnosis. An autopsy was performed 1 h after death.

Materials and methods

The autopsy liver specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin. Tissue sections, 4 μ m in thickness, were used for hematoxylin and eosin (HE) staining, special staining, and immunohistochemical examination. Primary antibodies used in this study are listed in Table 1. Detection was achieved with automated immunostainer (Ventana Medical System, Tucson, AZ, USA).

Ultrastructural analysis was performed for autopsied liver specimens which is fixed in 10% neutral-buffered formalin, postfixed in 2% osmium tetroxide, dehydrated, and embedded in epoxy resin. Ultrathin sections were examined under electron microscope (JEM1200EXII, Japan Electron Optics Laboratory, Tokyo, Japan).

DNA extracted from submicrodissected paraffin-embedded tissue was analyzed by polymerase chain reaction–single strand conformational polymorphism (PCR–SSCP) to screen for mutations in exons 5–8 of the *p53* gene. The primers used for PCR amplification were as follows: exon 5 (5'-TGTTCACTTGTCCTGACT-3' and 5'-CAGCCCTGTCGTCTCTCCAG-3'), exon 6 (5'-CCCAGGCCTCT

Table 1 Antibodies used for immunohistochemical studies and findings of both the adenocarcinoma and sarcomatoid components

Antibodies	Clone	Source	Dilution	Findings	
				Adenocarcinoma component	Sarcomatoid component
CK7	OV-TL 12/30	Dako, Glostrup, Denmark	1:50	+	P+
CK19	RCK108	Dako	1:50	+	+
CK7+8	CAM5.2	Becton-Dickinson, San Jose, CA, USA	Prediluted	+	+
Vimentin	V9	Dako	1:100	–	+
CA19-9	1116NS19-9	Cisbio international, Cèze Cedex, France	1:10	+	–
Hepatocyte	OCH1E5	Dako	1:25	–	–
Desmin	D33	Dako	1:100	–	–
Myoglobin	Polyclonal	Dako	1:9,000	–	–
Myogenin	F5D	Dako	1:100	–	–
MyoD1	5.8A	Dako	1:100	–	–
Mitochondria	AE-1	Leinco Technologies, St Louis, MO, USA	1:10	+	+
p53 protein	Bp53-12	IBL, Gunma, Japan	1:5000	+	–
E-cadherin	NCH-38	Dako	1:50	M+	W, C+
β -catenin	17C2	Novocastra, Newcastle upon Tyne, UK	1:100	W, C+	–
CD44s	DF1485	Novocastra	1:80	M+	M+
Ki-67	MIB-1	Dako	1:50	35.8%	49.7%

CK Cytokeratin, + positive, – negative, W weakly, P partially, M membranous, C cytoplasmic

GATTCCTCA-3' and 5'-CAACCACCCTTAACCCCTCC-3'), exon 7 (5'-ACTGGCCTCATCTTGGGCCT-3' and 5'-TGTGCAGGGTGGCAAGTGGC-3'), and exon 8 (5'-TAAATGGGACAGGTAGGACC-3' and 5'-TCCACCGCTTCTTGTCTGC-3'), respectively.

Results

At autopsy, the gross appearance of the tumor, which measured 4.0×2.8 cm, was reddish gray to tan in color with massive hemorrhagic necrosis (Fig. 1). Microscopically, the tumor was mostly necrotic and viable tumor cells were only identified in the periphery. The tumor was composed of two different components: moderately differentiated tubular adenocarcinoma and round cell elements (Fig. 2a). In the former portion, conglomerated atypical glands had proliferated with a fibrous stroma (Fig. 2b). In the latter portion, noncohesive round cells showed sarcomatoid appearances with eccentrically located nuclei and abundant eosinophilic cytoplasm (Fig. 2c). Multinucleated giant cells were scattered throughout the area (Fig. 2d). Round cells showed numerous lymphatic invasions into the liver and direct invasion into the gallbladder wall. No HCC component was detected. Lymph node metastasis, which was detected at the hepatic hilum and around the common bile duct, had resulted in obstructive jaundice. Metastatic nodules of round cells were identified in the bilateral adrenal glands. There was no evidence of cirrhosis, hepatolithiasis, or sclerosing cholangitis in the background liver. Mucin production of round cells was not detected in periodic acid Schiff (PAS), mucicarmine, and alcian blue stainings.

The immunohistochemical profiles of the two types of tumor cells are summarized in Table 1. The round cells were positive for cytokeratin (CK) 19 (Fig. 3a) and vimentin, but CK 7 expression was reduced. E-cadherin expression was lost at the membrane and weakly found in the cytoplasm



Fig. 1 Macroscopic appearance of the cut surface of the autopsied liver demonstrating a tumor of 4.0×2.8 cm in size, with hemorrhage and necrosis (arrowhead)

(Fig. 3b), while no immunoreactivity for β -catenin was detected (Fig. 3c). The round cells in the lymphatic vessels and stromal invasive sites demonstrated marked CD44s expression at the membrane (Fig. 3d). The tubular adenocarcinoma cells expressed CK19 (Fig. 3e) and β -catenin (Fig. 3g) in their cytoplasm, with E-cadherin (Fig. 3f) and CD44s (Fig. 3h) at the plasma membrane. Round cells and multinucleated giant cells presented immunoreactivity for mitochondria densely in the paranuclear cytoplasm. Nuclear expression of p53 protein was found in the adenocarcinoma cells. No specific expression of rhabdomyogenic markers was found for desmin, myoglobin, myogenin, or MyoD1. Electron micrographs revealed accumulation of large mitochondria in the cytoplasm of the round cells and multinucleated giant cells (Fig. 4). No band shifting was detected in exons 5–8 of the *p53* gene by PCR-SSCP analysis in both adenocarcinoma and round cell components.

Discussion

ISC is an uncommon variant of ICC and sarcomatoid component exhibited spindle-shaped cells in almost previously reported cases [5, 8, 15, 16, 20, 21]. Furthermore, ISC of round cell feature is very rare. Nakajima et al. [16] report a case of ICC comprised papillary carcinoma and round cells. Sasaki et al. [20] described a case of ISC composed of adenosquamous carcinoma, spindle-shaped cells, multinucleated giant cells, and round cells resembling rhabdomyosarcoma cells. Because the round cells of the present case apparently seemed to be rhabdomyosarcoma cells, displayed no mucin in their cytoplasm, and expressed both cytokeratins and vimentin, we considered this tumor as a sarcomatoid carcinoma. However, the round cells were not rhabdomyosarcoma or rhabdoid cells, as myogenic markers were not expressed by immunohistochemistry, and a whorl of intermediate filaments was not confirmed by ultrastructural study. Electron micrographs confirmed that the accumulation of mitochondria was attributable to the eosinophilic paranuclear cytoplasm. ICC with rhabdoid phenotype was reported by Honda et al. [7] and Lim et al. [12] but made no mention of ultrastructural findings.

Sarcomatous transformation occasionally appears in HCC cases treated with anticancer therapy, particularly, transcatheter arterial embolization (TAE) [11]. The present tumor was distinguished from HCC due to its negative immunoreactivity for an anti-hepatocyte antibody (OCH1E5). There have been no previous reports concerning the relationship between ISC and anticancer therapy. The previously reported cases of a round cell variant ICC and the present case had received neither TAE nor chemotherapy. It is, therefore, reasonable to consider that sarcomatoid transformation is a biologically natural

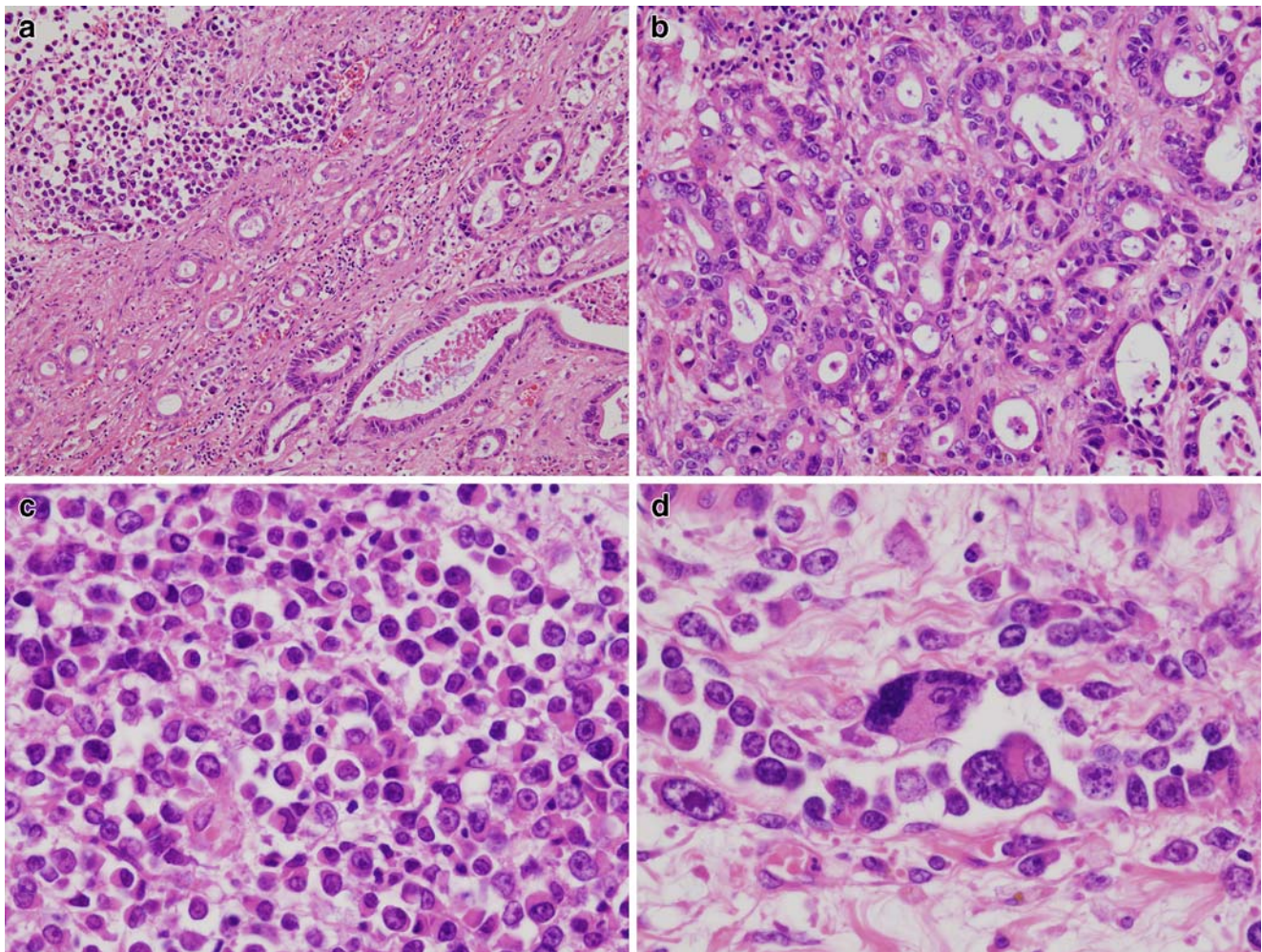


Fig. 2 Histological appearances of the tumor (Hematoxylin and eosin staining). **a** Tubular adenocarcinoma (*lower right*) and sarcomatoid components with round feature (*upper left*) are observed in the periphery, $\times 100$. **b** Tubular adenocarcinoma composed of small

atypical glands, $\times 200$. **c** Sarcomatoid round cells are noncohesive and have eccentric large vesicular nuclei, prominent nucleoli, and abundant paranuclear eosinophilic cytoplasm, $\times 400$. **d** Multinucleated giant cells are shown, $\times 400$

course of tumor progression rather than a secondary event due to artificial treatment.

In ISC, sarcomatoid component usually coexists with cholangiocarcinoma component. The previously reported cases of ISC showed cytokeratin expressions in both the adenocarcinoma and sarcomatoid components [5–7, 9, 15, 20, 21]. In the present case, immunohistochemical positivity for CK19, which is restricted to the biliary epithelium within the liver, was detected in both the adenocarcinoma component and round cells. Therefore, the round cell component can reasonably be considered to have transformed from the cholangiocarcinoma cells. Differences in the immunoreactivities for CK7, vimentin, and CA19-9 between adenocarcinoma and round cells suggest that alterations in genetic expressions and phenotypic changes occur during sarcomatoid transformation. By immunohistochemistry, the expression of p53 protein was different between adenocarcinoma and round cells, but no mutation

was detected in exons 5–8 of the *p53* gene in both components.

Normal bile duct epithelial cells show immunoreactivities for E-cadherin and β -catenin on the plasma membrane [2]. Reductions of these molecules were detected in cholangiocarcinoma and correlated with high-grade character [2]. The expressions of E-cadherin and β -catenin contribute to the maintenance of the normal morphology and polarity of bile ducts [2]. In the present case, immunohistochemical staining for E-cadherin revealed diffuse membranous expression in the adenocarcinoma component and weak cytoplasmic expression in the round cell component. The β -catenin immunoreactivity was weakly positive in the adenocarcinoma component and negative in the round cells. Loss of the membranous expression and cytoplasmic localization of E-cadherin have been reported to be associated with functional and structural disorders [22]. Loss of the membranous expres-

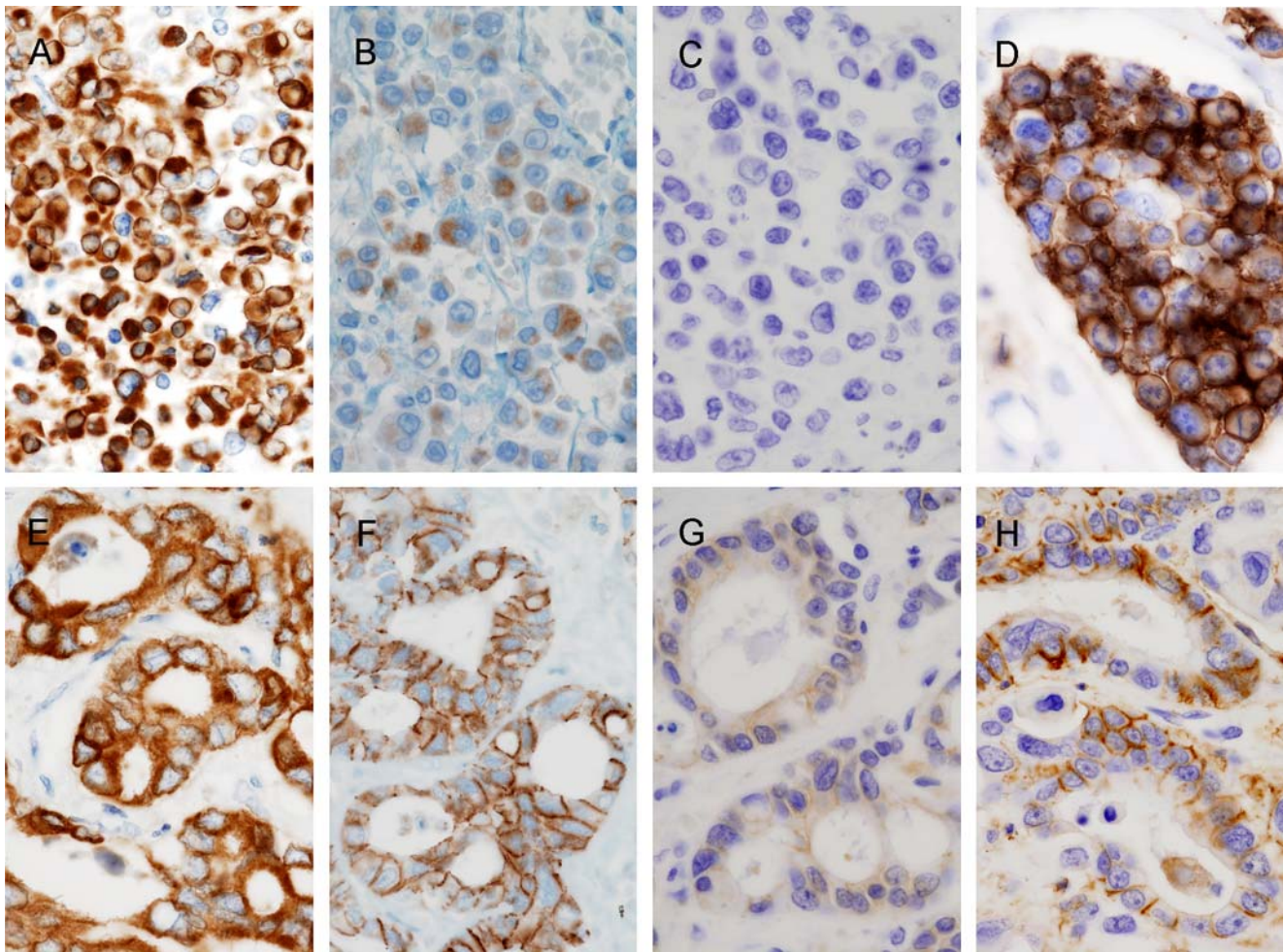


Fig. 3 Immunohistochemical findings of the tumor cells, $\times 400$. The round cells express CK19 in the cytoplasm (a) and E-cadherin weakly in the cytoplasm (b), but no β -catenin expression is detected (c). The round cells in the lymph vessels express CD44s at the plasma

membrane (d). The tubular adenocarcinoma cells express CK19 in the cytoplasm (e), E-cadherin at the membrane around the cell (f), β -catenin weakly in the cytoplasm (g) and CD44s at the lateral plasma membrane (h)

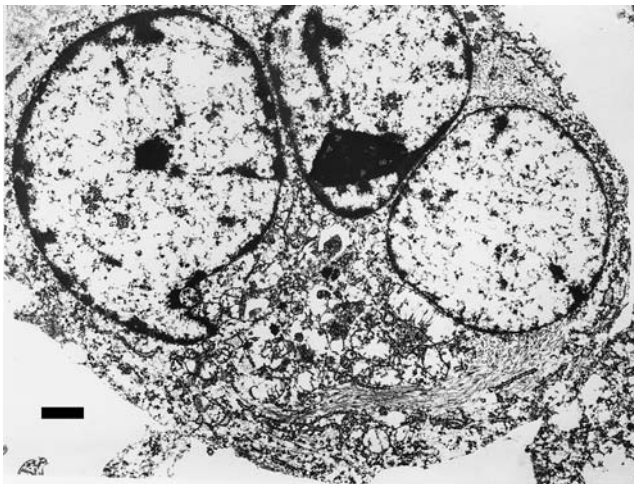


Fig. 4 Electron microscopic findings of multinucleated giant cells. Large mitochondria are accumulated in cytoplasm. Scale bar=1 μm

sions of E-cadherin and β -catenin may play an important role in disruption of the histologic architecture and aggressive behavior for invasion and metastasis of the round cell component.

CD44s is aberrantly expressed at the membrane of cholangiocarcinoma cells [2]. However, its roles in tumor invasion and lymph-vascular penetration have not been fully elucidated. In HCC, upregulation of CD44 was related to vascular invasion [13]. In contrast, reduced or loss of CD44 expression is associated with lymph-vascular permeation in invasive micropapillary carcinoma of the breast, endometrial carcinoma, and colon carcinoma [1, 3, 4]. In the present case, the round cells showed a distinct membranous expression of CD44s. The CD44s immunoreactivity was more intense in the round cells than in the adenocarcinoma cells. The results suggest that aberrant overexpression of CD44s may be involved in marked

lymph–vascular invasion by sarcomatoid cholangiocarcinoma cells as has been reported for HCC.

In conclusion, ISC with a round cell phenotype is an aggressive variant of ICC. Loss or reduction of the membranous expressions of E-cadherin and β -catenin and upregulation of CD44s in the round cell component may be associated with the high propensity for lymph–vascular permeation.

Acknowledgements The authors thank Ms. Yumi Tsubata (Department of Pathology, Kanazawa Medical University Hospital) for immunohistochemistry and Ms. Yoshiiku Okanemasa (Department of Pathology, Kanazawa Medical University Hospital) for electron micrographs.

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