Pathol. Oncol. Res. (2009) 15:533–539
DOI 10.1007/s12253-009-9150-4

ORIGINAL PAPER

Zonula Occludens-1, Occludin, and E-cadherin Protein Expression in Biliary Tract Cancers

Zsuzsanna Németh · Attila Marcell Szász · Áron Somorácz · Péter Tátrai · Júlia Németh · Hajnalka Győrffy · Attila Szíjártó · Péter Kupcsulik · András Kiss · Zsuzsa Schaff

Received: 4 November 2008 / Accepted: 8 January 2009 / Published online: 2 February 2009

Abstract The incidence of cholangiocarcinomas originating from intrahepatic and extrahepatic bile ducts, as well as of gallbladder carcinoma is increasing worldwide. The malignant transformation of biliary epithelia involves profound alterations of proteins in the intercellular junctions, among others zonula occludens-1 (ZO-1), occludin, and E-cadherin. Each plays important role in the maintenance of epithelial cell polarity and regulation of cell growth and differentiation. Our aim was to investigate ZO-1, occludin, and E-cadherin immunohistochemical reactions in tissue microarray blocks containing 57 normal and 62 neoplastic biliary tract samples. We demonstrated that the tight junction components ZO-1, occludin, and E-cadherin are downregulated in carcinomas arising from various compartments of the biliary tract (normal intrahepatic and extrahepatic bile ducts, gallbladder) as compared with their normal sites of origin. These results were confirmed by discriminant analysis yielding clear separation of the three normal sample groups from carcinomas in the corresponding locations.

Keywords Adherens junction · Biliary tract cancer · Tight junction

Németh and Szász contributed equally to this work

Z. Németh · A. M. Szász · Á. Somorácz · P. Tátrai · J. Németh · H. Győrffy · A. Kiss · Z. Schaff (✉)
2nd Department of Pathology, Semmelweis University,
Üllői út 93,
H-1091 Budapest, Hungary
e-mail: schaff@korb2.sote.hu

A. Szíjártó · P. Kupcsulik
1st Department of Surgery, Semmelweis University,
Budapest, Hungary

Abbreviations

CCCs	Cholangiocarcinomas
GBCs	gallbladder carcinomas
EBDC	extrahepatic bile duct cancers
IBDC	intrahepatic bile duct cancers
AJ	adherens junctions
TJ	tight junctions
ZO-1	zonula occludens-1
EMT	epithelial-to-mesenchymal transition
FFPE	formalin-fixed, paraffin-embedded
TMA	Tissue Micro-Array
NIBD	normal intrahepatic bile duct
NEBD	normal extrahepatic bile duct
NGB	normal gall bladder

Introduction

Cholangiocarcinomas (CCCs) originating from intrahepatic and extrahepatic bile ducts as well as gallbladder carcinomas (GBCs) predominantly occur in patients over 55–60 years [1, 2]. CCCs affect both genders, the risk factors being primary sclerosing cholangitis, alcohol abuse, smoking, viral infection and, in rare instances, ulcerative colitis [1, 3, 4]. The majority (70–80%) of CCCs are extrahepatic bile duct cancers (EBDC) originating from the extrahepatic portion of the biliary tree [1]. The rest are comprised of intrahepatic bile duct cancers (IBDC) which are becoming more and more frequent in both the Western countries and Japan [5]. GBC—primarily affecting women—is among the most common malignancies of the gastrointestinal tract. Previous studies have failed to clarify the role of gallstones in gallbladder carcinogenesis [1, 6]. A high risk factor for

the development of all biliary tract cancers including CCCs as well as GBCs is pancreatobiliary maljunction [7].

Despite the rising incidence of biliary tract cancers worldwide, very little is known about the cellular alterations of the biliary epithelium that accompany carcinogenesis. During malignant transformation of epithelia in general, intercellular junctions undergo significant changes in structure [8–10]. The junctional complexes: adherens junctions (AJ), tight junctions (TJ), and desmosomes connect the epithelial cells to each other. While E-cadherin is one of the main components of AJs, occludin (a transmembrane protein) and zonula occludens-1 (ZO-1) (a plaque protein) are located in TJs [9]. Along with AJs and desmosomes, TJs also act as guardians of epithelial cell polarity [11, 12]. The TJ membrane protein complex constitutes a barrier against paracellular diffusion of solutes, and restricts lateral diffusion of the lipid and protein components of the cell membrane [13–16]. Moreover, TJs were observed to regulate growth and differentiation of cultured cells [17, 18] and to play role in epithelial-to-mesenchymal transition (EMT) [8]. The loss of E-cadherin has also been implicated in cancer progression and EMT. The lack of this component from AJs correlates with poor prognosis in breast cancer patients [19, 20].

The current study focused therefore on comparison of the immunostaining profile of ZO-1, occludin, and E-cadherin in the normal biliary tract and bile duct carcinomas, in order to investigate whether the carcinogenesis process and tumor progression are associated with expression changes of these proteins.

Patients and Methods

Patient Material

We investigated a set of surgically removed, formalin-fixed, paraffin-embedded (FFPE) bile duct carcinomas from 62 patients (Table 1) which were analyzed for ZO-1, occludin, and E-cadherin expression. Control specimens were selected from 12 intrahepatic bile duct samples from portal tracts, 12 normal extrahepatic bile duct samples, and 33 normal gallbladder samples. Patients did not receive chemotherapy or radiotherapy prior to surgery. The median age was 65 and the male-female ratio was 24/38. Cancer samples were classified by routine diagnostic procedure according to the Cancer Grading Manual criteria, graded as well-differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3) tumors (Table 1) [21]. Glandular structures of varying sizes were present in well- and moderately differentiated (G1-2) specimens, while poorly differentiated (G3) cases frequently showed solid growth pattern with small- to medium-sized cells. Acute inflammation was not noted in the tissue specimens. The study was approved by the Regional Ethical Committee (#172/2003).

Table 1 Classification of biliary tract samples according to cancer grading manual (See ref. [21])

Groups	G1	G2	G3	Total
IBDC	1	6	4	11
EBDC	0	12	5	17
GBC	1	19	14	34

Numbers of samples in each group according to grade
IBDC intrahepatic bile duct cancer, *EBDC* extrahepatic bile duct cancer, *GBC* gallbladder cancer

Tissue Microarray

In all cases, hematoxylin-eosin-stained slides were used to select representative tumor areas. The area of interest in the donor block was cored twice with a 2.0 mm diameter needle and transferred into a recipient block with a total capacity of 24 cores. The instrument used for the procedure was a Tissue Micro-Array (TMA) Builder (Histopathology Ltd., Pécs, Hungary). Multiblocks were incubated twice for 5 min at 56°C to improve adhesion between cores and paraffin of the recipient block. Cores from 62 tumor and 57 normal sample donor blocks were placed in 15 TMA recipient blocks (3=normal intrahepatic bile duct (NIBD) +normal extrahepatic bile duct (NEBD)+normal gall bladder (NGB), 3 NGBs, 2 IBDCs, 1 EBDC, 3 GBCs, and 3 mixed from the cancer groups). Each TMA block contained duplicates or triplicates of the selected samples and 2–3 controls (corresponding tumor and normal samples for normal and tumor blocks, respectively, and hepatocellular carcinoma for all). Morphology of the selected tissues was controlled on 3–4 μm thick whole TMA-sections after hematoxylin-eosin staining.

Immunohistochemistry

Expression of ZO-1, occludin, E-cadherin and cytokeratin-7 proteins was analyzed by immunohistochemistry. Immunohistochemical analysis of the different tumor groups was performed by use of the antibodies and conditions shown in Table 2. The immunohistochemical reactions were performed on 3–4 μm thick FFPE sections obtained from the TMA blocks. After the deparaffination steps, slides were washed in PBS (pH 7.4), then treated for 30 min in a retrieval solution (Target Retrieval Solution cat# S1699 from DAKO, Glostrup, Denmark) in a microwave oven. In addition, samples were digested with 0.1 mg/mL pronase E (Sigma) for 5 and 3 min for optimal detection of ZO-1 and occludin, respectively. Reactions were carried out in Ventana ES automated immunostainer (Ventana Medical

Table 2 Primary antibodies

	Dilution	Positive control	Host and clonality	Company	Catalogue No.
ZO-1	1:100	normal small intestine	Rabbit polyclonal	Zymed	40-2300
Occludin	1:100	normal small intestine	Rabbit polyclonal	Zymed	71-1500
E-cadherin	1:120	Mammary ductal cc.	Mouse monoclonal	DAKO	M3612
CK 7	1:300	normal bile ducts	Mouse monoclonal	DAKO	M7018

(Zymed Inc, San Francisco, CA, USA; DAKO, Glostrup, Denmark)
ZO-1 zonula occludens-1

Systems Inc., Tucson, AZ, USA). Reagents and secondary antibody from the Ventana detection kit (iView DAB Detection Kit, cat# 760-091, Ventana) were used according to manufacturer's instructions. Negative and positive controls were included for all antibodies. The positive controls are shown in Table 2.

Semiquantitative Immunohistochemical Analysis

Immunohistochemical reactions were photodocumented using Mirax Midi Scanner (3DHISTECH, Budapest, Hungary). All cases were evaluated from two aspects. Intensity of immunoreactions was described using three grades: 1 - weakly positive, 2 - moderately positive and 3 - strongly positive. The value of positivity was measured in all cases as percentage of positive cells in the whole representative area.

Statistical Analysis

Statistical analysis was performed using SPSS 15.0 software (SPSS Inc., Chicago, Ill, USA). The Mann-Whitney test was performed to compare protein expression of the selected sample group pairs (NIBD-IBDC, NEBD-EBDC, NGB-GBC, IBDC-EBDC, IBDC-GBC, and EBDC-GBC). The Bonferroni-Holmes test was used as an additional test for corrections. The p values less than 0.05 were considered to be statistically significant. Correlation between intensity and immunopositivity of an area was calculated by Spearman's rank correlation; $p<0.01$ was defined as significant difference between the studied variables. SPSS 15.0 software (SPSS Inc., Chicago, Ill, USA) was used to demonstrate the distinction of groups by discriminant analysis.

Results

Characterization of ZO-1, Occludin and E-cadherin Immunoreaction Patterns ZO-1 and occludin showed plasma membrane immunoreaction pattern on the apical side of the membrane only, whereas E-cadherin exhibited both membranous and cytoplasmic reactions. While the immunoreactions remained apical in acinary structures of well-

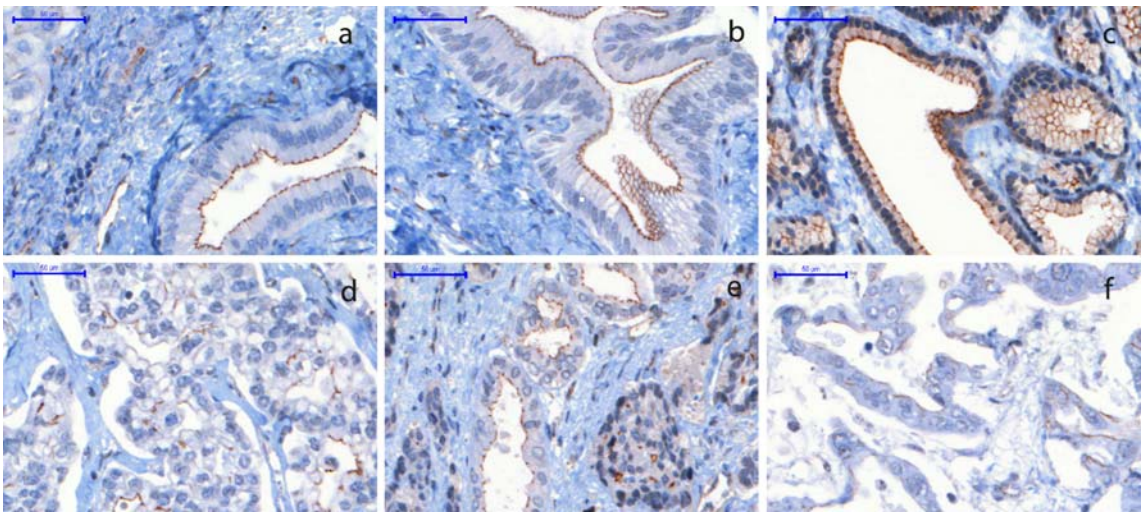

Fig. 1 ZO-1 immunoreactions in the NIBD (**a**), NEBD (**b**), NGB (**c**), IBDC (**d**), EBDC (**e**), and GBC (**f**) groups. ZO-1 protein expression was significantly decreased in the carcinoma samples as compared with their corresponding normal regions. Significant differences were rather seen in the percentage of positive cells than in the intensity of immunoreactions (NIBD=normal intrahepatic bile duct; NEBD=normal extrahepatic bile duct; NGB=normal gallbladder; IBDC=intrahepatic bile duct cancer; EBDC=extrahepatic bile duct cancer; GBC=gallbladder cancer). Bars: 0.05 mm

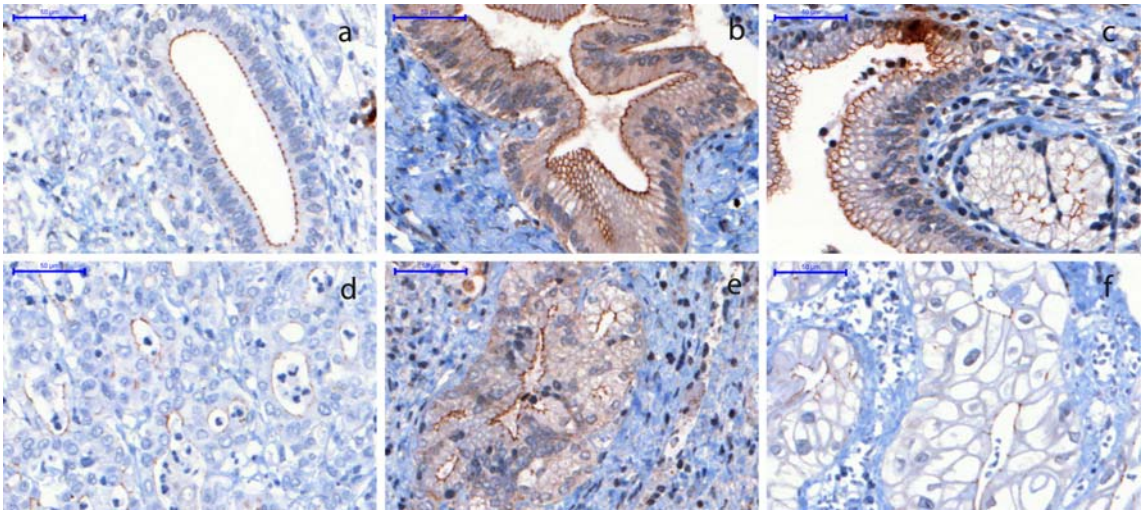

Fig. 2 Occludin immunoreactions in the NIBD (**a**), NEBD (**b**), NGB (**c**), IBDC (**d**), EBDC (**e**), and GBC (**f**) groups. The proportion of occludin-positive cells was significantly lower in the cancer samples when compared with corresponding normal adjacent tissues (NIBD=normal intrahepatic bile duct; NEBD=normal extrahepatic bile duct; NGB=normal gallbladder; IBDC=intrahepatic bile duct cancer; EBDC=extrahepatic bile duct cancer; GBC=gallbladder cancer). Bars: 0.05 mm

differentiated tumors, aberrant diffuse membrane localization of ZO-1 and occludin was observed on small- to medium-sized cells in higher-grade tumors with solid growth pattern. Unlike normal samples where all biliary epithelial cells expressed the investigated proteins either moderately or strongly, fewer or no cells exhibited positive reactions in the carcinoma samples.

Comparison of ZO-1, Occludin, E-cadherin, and Cytokeratin-7 Protein Expression Between Sample Groups Significant differences were found in normal vs. carcinoma comparisons (see Figs. 1, 2 and 3), unlike comparisons made within either the normal or neoplastic sample groups. The intensities and positivities of immunoreactions showed significant correlation in all cases. The results of semiquantitative analysis are shown in Table 3. Regarding all three proteins studied, positivity percentages of tumor groups were significantly lower than for the corresponding normal groups, with ZO-1 showing the most pronounced decrease in the rate of positive cells in tumors. In addition, reaction intensity of E-cadherin was also significantly weaker when comparing GBC and NGB. Other differences in intensity failed to demonstrate any significance. Cytokeratin-7 showed strong positivity in all

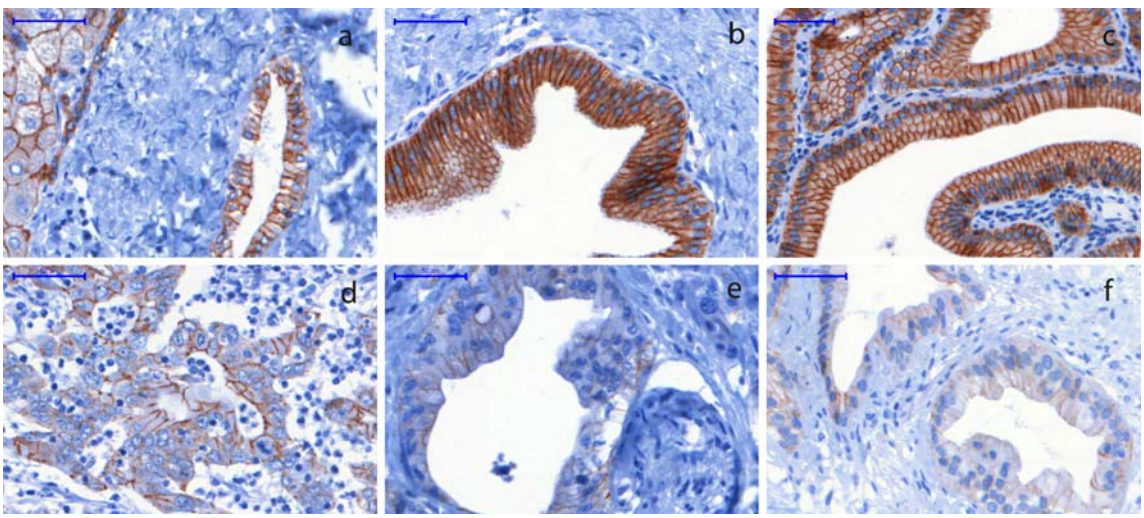

Fig. 3 E-cadherin immunoreactions in the NIBD (**a**), NEBD (**b**), NGB (**c**), IBDC (**d**), EBDC (**e**), and GBC (**f**) groups. Significant decrease was seen in the percent ratio of positive cells (and, in case of GBC and NGB, also in the intensity of immunoreaction) in the tumor samples relative to the corresponding normal tissues (NIBD=normal intrahepatic bile duct; NEBD=normal extrahepatic bile duct; NGB=normal gallbladder; IBDC=intrahepatic bile duct cancer; EBDC=extrahepatic bile duct cancer; GBC=gallbladder cancer). Bars: 0.05 mm

Table 3 Semiquantitative analysis of ZO-1, occludin and E-cadherin protein expression in biliary tract samples

	Var.	NIBD-IBDC	NEBD-EBDC	NGB-GBC	IBDC-EBDC	IBDC-GBC	EBDC-GBC	correlation coefficient (*p* value)
ZO-1	int	ns	ns	ns	ns	ns	ns	0.379 **(<0.001)**
	%	**<0.001**	**0.001**	**<0.001**	ns	ns	ns	
Occl	int	ns	ns	ns	ns	ns	ns	0.566 **(<0.001)**
	%	**0.008**	**0.02**	**<0.001**	ns	ns	ns	
E-cadh	int	ns	ns	**<0.001**	ns	ns	ns	0.496 **(<0.001)**
	%	**<0.001**	**0.001**	**<0.001**	ns	ns	ns	

Groups were compared by the Mann-Whitney test (significant if $p<0.05$) from the aspects of immunoreaction intensity (*int*) and percentage of immunopositive cells (*%*) further, correlation (significant if $p<0.01$) was calculated between these two variables
Groups: *NIBD* normal intrahepatic bile duct, *NEBD* normal extrahepatic bile ducts, *NGB* normal gallbladder, *IBDC* intrahepatic bile duct cancer, *EBDC* extrahepatic bile duct cancer, *GBC* gallbladder cancer, *ZO-1* zonula occludens-1, *Occl* occluding, *E-cadh* E-cadherin, *ns* non significant

normal samples, contrary to the three carcinoma groups where decreased expression of this protein was notable in poorly differentiated regions of epithelial carcinomas (results not shown).

Discriminant analysis based on both positivity and intensity data of the immunoreactions of the three proteins resulted in the sharp separation of normal groups (NIBD, NEBD, NGB) from tumor samples (carcinomas in corresponding locations) (Fig. 4).

Comparison of Cancer Groups and Subgroups The selected cancer groups did not differ significantly from each other in any aspect of evaluation (Table 3, Fig. 4). In all cancer groups, approximately two thirds of tumor cells showed positive occludin and E-cadherin immunoreaction, whereas one third exhibited only ZO-1 immunopositivity. E-cadherin showed the strongest reactions in all cancer groups. The positivity rates decreased with the grade in all cancer groups, however, statistical analysis could not be applied due to the paucity of G1 samples.

Discussion

The role of AJ and TJ proteins in cell polarity, growth and differentiation has been described by several authors [9, 10, 22]. AJs and TJs have also been implicated in cell proliferation and cancer [8, 20, 23, 24]. Previous studies have focused on TJ plaque proteins which play direct role in cell motility by participating in the rearrangement of the actin cytoskeleton [8]. Moreover, TJ components possess the ability to modulate epithelial permeability barrier properties by influencing the passage of cations and—to a lesser extent—anions. For instance, insertion of the integral TJ component occludin into the plasma membrane in its phosphorylated form leads to an increase in transepithelial resistance [9]. On the other hand, loss or decreased expression of AJ and TJ proteins may bring about disturbances in cell polarity, thereby modifying the distribution of lipid rafts and their protein components (receptors, channels etc.). The consequent expansion of the signal reception field may have an impact on the responsiveness of epithelial cells to extracellular signals [8, 10].

The present report has demonstrated a decrease in expression of TJ components ZO-1, occludin, and E-cadherin in biliary tract cancers as compared with their

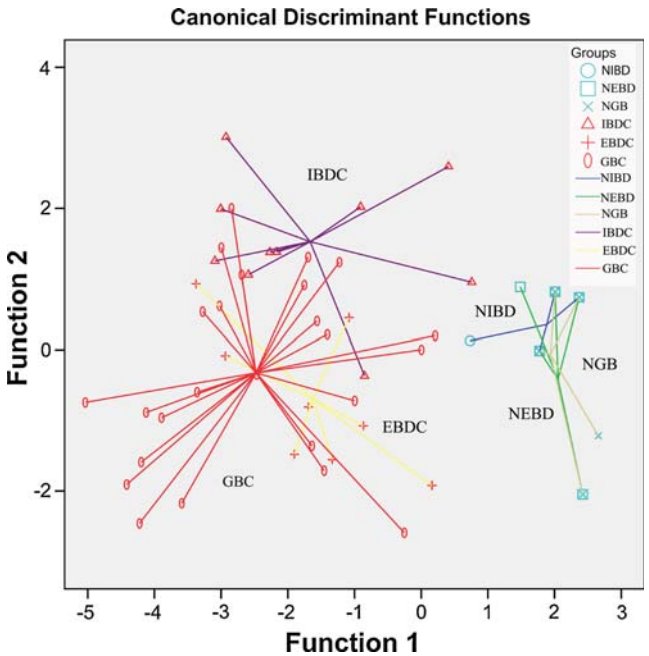

Fig. 4 Discriminant analysis. The three normal groups (NIBD= normal intrahepatic bile duct, NEBD=normal extrahepatic bile duct, NGB=normal gallbladder) and three cancer groups (IBDC= intrahepatic bile duct cancer, EBDC=extrahepatic bile duct cancer, and GBC= gallbladder cancer) are sharply separated by discriminant analysis based on the immunoreactions of ZO-1, occludin and E-cadherin, whereas both the three cancer and three normal groups cluster together. (Lines point at the centroid of each group.) The number of symbols in the graph is smaller than the total number of cases due to the occasional overlap of data points

originating tissues. This decrease was manifested as a lower percentage of immunoreactive cells rather than as an overall drop of immunostaining intensity. Specifically, the number of ZO-1-positive cells decreased significantly in the tumor samples relative to their normal adjacent tissues, and the localization of immunoreaction was altered in the less-differentiated tumors, appearing on the entire circumference of cells. ZO-1 expression was also reported to be decreased in non-malignant pathological conditions of acute calculous and acalculous cholecystitis [25]. By contrast, Kleeff and colleagues reported an inverse sequence of changes in ZO-1 protein expression in the pancreas: the faintest positivity was detected in normal human pancreatic tissues, with moderate expression recorded in chronic pancreatitis and moderate to high expression in all pancreatic cancer samples [26]. However, these authors observed the same dependence of ZO-1 immunolocalization on the degree of tumor differentiation: in cases showing duct-like or ductular structures the reactions were present on the apical portion of the cell membrane, as opposed to solid growth pattern tumors (higher grade cancers with lymph node metastases) in which cases diffuse membranous staining was found.

Parallel to ZO-1, the rate of immunopositive cells for occludin was also significantly decreased in all cancers relative to the corresponding normal sites of origin. The higher permeability of TJ structure caused by the diminished expression of occludin may contribute to EMT, consequently opening gates for tumor progression [27–29]. Similarly, E-cadherin immunopositivity rates were significantly lower in the IBDC, EBDC, and GBC groups as compared with the corresponding normal sample groups (NIBD, NEBD, NGB). Farazi and co-workers, as well as Okada and colleagues reported decreased E-cadherin expression in intrahepatic CCC and EBDC [30, 31]. The loss or reduced membrane positivity of E-cadherin may be a potential prognostic marker in CCC and GBC: in these cancers the 5-year survival rate is significantly lower in cases with reduced E-cadherin compared with the cases showing preserved E-cadherin expression [32, 33]. Diminished E-cadherin expression was found to be significantly associated with poor tumor cell differentiation and inferior survival by Park and co-workers [34]. Berx and colleagues have reported that the partial or complete loss of E-cadherin correlates with poor prognosis in breast cancer [20]; similarly, Cowin and co-workers describe an irreversible loss of E-cadherin expression in invasive lobular breast cancer [19].

While the expression changes of TJ proteins related to biliary tract carcinogenesis have formerly not been investigated, Laurila and co-workers detected an inflammation-related decrease in ZO-1 and occludin immunopositivity when comparing NGBs with acute calculous and acalculous cholecystitis. In agreement with our findings, their data suggest that out of a panel of TJ proteins including ZO-1, occludin, and E-cadherin, it was the expression of ZO-1 that underwent the most pronounced downregulation in disease [25]. The expression of occludin, too, reacted with downregulation to the condition of acute cholecystitis, while no differences in E-cadherin expression were found between normal and inflamed gallbladder [25].

Taken together, this is the first study to compare the protein expression of ZO-1 and occludin in normal versus neoplastic biliary tracts, although a previous study carried out in our department found strong ZO-1 and occludin immunoreactions in case of normal bile canaliculi [35]. The present report demonstrated that TJ components ZO-1, occludin, and E-cadherin are downregulated in carcinomas arising from various compartments of the biliary tract (NIBDs, NEBDs, gallbladder) as compared with their normal sites of origin. These results were confirmed by discriminant analysis which yielded clear separation of the three normal sample groups from carcinomas in the corresponding locations. Further investigations are needed to clarify the role of altered TJ structures in the progression of biliary tract cancers, as well as the potential prognostic significance of the observed expression changes.

Acknowledgements Authors wish to thank Mrs Magdolna Pekár and Mrs Erzsébet Azumah for preparing the TMAs and the immunohistochemical reactions and Mrs Elvira Kale-Rigo for the careful reading and correction of the manuscript.

This work was supported by Grant 75468 from the Hungarian Scientific Research Fund (OTKA).

Conflict of interest statement The authors of the present study confirm that there is no conflict of interest to be declared.

References

1. Kakar S, Ferrell LD (2007) Tumors of the liver, gallbladder and biliary tree. In: Fletcher CDM (ed) Diagnostic histopathology of tumors, 3rd edn. Elsevier Limited, Philadelphia, pp 417–462
2. Chuang SC, Lee KT, Tsai KB, et al (2004) Immunohistochemical study of DPC4 and p53 proteins in gallbladder and bile duct cancers. World J Surg 28:995–1000
3. Bajor J, Bero T, Garamszegi M, et al (2001) Common bile duct tumor in a young woman with ulcerative colitis. Orv Hetil 142:1231–1234
4. Hong SM, Kim MJ, Pi DY, et al (2005) Analysis of extrahepatic bile duct carcinomas according to the New American Joint Committee on Cancer staging system focused on tumor classification problems in 222 patients. Cancer 104:802–810
5. Obama K, Ura K, Li M, et al (2005) Genome-wide analysis of gene expression in human intrahepatic cholangiocarcinoma. Hepatology 41:1339–1348
6. Lewis JT, Talwalkar JA, Rosen CB, et al (2007) Prevalence and risk factors for gallbladder neoplasia in patients with primary sclerosing cholangitis: evidence for a metaplasia-dysplasia-carcinoma sequence. Am J Surg Pathol 31:907–913
7. Kobayashi S, Ohnuma N, Yoshida H, et al (2006) Preferable operative age of choledochal dilation types to prevent patients with pancreaticobiliary maljunction from developing biliary tract carcinogenesis. Surgery 139:33–38

8. Gonzalez-Mariscal L, Lechuga S, Garay E (2007) Role of tight junctions in cell proliferation and cancer. Prog Histochem Cytochem 42:1–57
9. Hartsock A, Nelson WJ (2008) Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. Biochim Biophys Acta 1778(3):660–669
10. Schneeberger EE, Lynch RD (2004) The tight junction: a multifunctional complex. Am J Physiol Cell Physiol 286: C1213–C1228
11. Rodriguez-Boulan E, Nelson WJ (1989) Morphogenesis of the polarized epithelial cell phenotype. Science 245:718–725
12. Miyoshi J, Takai Y (2005) Molecular perspective on tight-junction assembly and epithelial polarity. Adv Drug Deliv Rev 57:815–855
13. Gumbiner B (1987) Structure, biochemistry, and assembly of epithelial tight junctions. Am J Physiol 253:C749–C758
14. Gumbiner BM (1993) Breaking through the tight junction barrier. J Cell Biol 123:1631–1633
15. Anderson JM, Van Itallie CM (1995) Tight junctions and the molecular basis for regulation of paracellular permeability. Am J Physiol 269:G467–G475
16. Ivanov AI, Nusrat A, Parkos CA (2005) Endocytosis of the apical junctional complex: mechanisms and possible roles in regulation of epithelial barriers. Bioessays 27:356–365
17. Tsukita S, Furuse M, Itoh M (1999) Structural and signalling molecules come together at tight junctions. Curr Opin Cell Biol 11:628–633
18. Balda MS, Matter K (1998) Tight junctions. J Cell Sci 111(Pt 5):541–547
19. Cowin P, Rowlands TM, Hatsell SJ (2005) Cadherins and catenins in breast cancer. Curr Opin Cell Biol 17:499–508
20. Berx G, van Roy F (2001) The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression. Breast Cancer Res 3:289–293
21. Guzman G, Chejfec G (2007) Tumors of the digestive system. In: Damjanov I, Fan F (ed) Cancer grading manual. Springer, New York, pp 43–44
22. Umeda K, Ikenouchi J, Katahira-Tayama S, et al (2006) ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. Cell 126:741–754
23. Hugh TJ, Dillon SA, Taylor BA, et al (1999) Cadherin-catenin expression in primary colorectal cancer: a survival analysis. Br J Cancer 80:1046–1051
24. Truant SC, Gouyer VP, Leteurtre EA, et al (2008) E-Cadherin and beta-Catenin mRNA Levels Throughout Colon Cancer Progression. J Surg Res 2008 Feb 11 [Epub ahead of print]
25. Laurila JJ, Karttunen T, Koivukangas V, et al (2007) Tight junction proteins in gallbladder epithelium: different expression in acute acalculous and calculous cholecystitis. J Histochem Cytochem 55:567–573
26. Kleeff J, Shi X, Bode HP, et al (2001) Altered expression and localization of the tight junction protein ZO-1 in primary and metastatic pancreatic cancer. Pancreas 23:259–265
27. Medici D, Hay ED, Goodenough DA (2006) Cooperation between snail and LEF-1 transcription factors is essential for TGF-beta1-induced epithelial-mesenchymal transition. Mol Biol Cell 17:1871–1879
28. Kurrey NK, K A, Bapat SA (2005) Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. Gynecol Onco l97:155–165
29. Ikenouchi J, Matsuda M, Furuse M, et al (2003) Regulation of tight junctions during the epithelium-mesenchyme transition: direct repression of the gene expression of claudins/occludin by Snail. J Cell Sci 116:1959–1967
30. Okada K, Shimura T, Asakawa K, et al (2007) Fascin expression is correlated with tumor progression of extrahepatic bile duct cancer. Hepatogastroenterology 54:17–21
31. Farazi PA, Zeisberg M, Glickman J, et al (2006) Chronic bile duct injury associated with fibrotic matrix microenvironment provokes cholangiocarcinoma in p53-deficient mice. Cancer Res 66:6622–6627
32. Hirata K, Ajiki T, Okazaki T, et al (2006) Frequent occurrence of abnormal E-cadherin/beta-catenin protein expression in advanced gallbladder cancers and its association with decreased apoptosis. Oncology 71:102–110
33. Settakorn J, Kaewpila N, Burns GF, et al (2005) FAT, E-cadherin, beta catenin, HER 2/neu, Ki67 immuno-expression, and histological grade in intrahepatic cholangiocarcinoma. J Clin Pathol 58:1249–1254
34. Park S, Kim SW, Lee BL, et al (2006) Expression of E-cadherin and beta-catenin in the adenoma-carcinoma sequence of ampulla of Vater cancer. Hepatogastroenterology 53:28–32
35. Orban E, Szabo E, Lotz G, et al (2008) Different expression of Occludin and ZO-1 in primary and metastatic liver tumors. Pathol Oncol Res 14:299–306