Chapter 14

Simultaneous Changes in Expression of Bile Canalicular CD10 and Sinusoidal CD105 (Endoglin) in Chronic Hepatitis and Liver Cirrhosis

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Additional information is available at the end of the chapter

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1. Introduction

Liver tissue contains hepatic sinusoids and bile canaliculi among hepatocyte plates. Dynamic changes of these components during a sequence of chronic hepatitis (CH) to liver cirrhosis (LC) have been an interesting subject from a standpoint of cellular injury and regeneration. It is known that proliferative activity of hepatocytes is significantly correlated with the severity of CH [1-3]. On the other hand, the dynamic changes of proliferative sinusoidal cells have been analyzed in experimental liver injury [4,5], but not enough in human cases of CH/LC in relation to inflammatory activity or fibrosis stage. Similarly, bile canalicular changes in CH/LC have not been analyzed until recently, because the bile canaliculi are difficult to recognize morphologically.

Recent identification of markers for bile canaliculi or hepatic sinusoids facilitated the analysis of phenotypic changes in these components in CH/LC. Some markers for bile canaliculi, such as CD10, CD13 and biliary glycoprotein I, are known at present [6,7]. CD10, also called common acute lymphoblastic lymphoma antigen (CALLA) or neprilysin, is a 100kD type II cell-surface metalloproteinase [8] and modulates the enkephalin-mediated inflammatory response [9]. It is expressed in various tissues, including brush border of enterocytes, renal tubules/glomeruli, endometrial stroma, hepatic bile canaliculi and lymphoid precursor cells [10]. It is also a useful marker for neoplastic counterpart of these tissues such as some lymphoma/leukemia [11], renal cell carcinoma, endometrial stromal sarcoma [12] and hepatocellular carcinoma (HCC) [6,7,13,14]. However, changes of expression pattern of CD10 in bile canaliculi (CD10(BC)) during a sequence of CH/LC have not been examined except for a report by Shousha *et al.* [15]

CD105, also known as endoglin, is an 180kD homodimeric transmembrane glycoprotein forming part of transforming growth factor-β (TGF-β) receptor complex [16,17]. It is expressed with marked tissue-specificity, predominantly in vascular endothelial cells of tissues undergoing active angiogenesis such as regenerating or inflamed tissue and tumoral stroma [18,19]. In particular, its expression in the stromal vessels of various carcinomas is associated with unfavorable prognosis [19-22]. Thus, CD105 has been attracted considerable attention, not only as a biological marker of tumor growth but also as a target molecule for diagnostic and therapeutic application against cancer [23,24]. On the other hand, CD105 expression has also been examined in the active/proliferative vessels of stromal tissues other than carcinomas, such as hemorrhoids [25], inflammation and wound healing [26,27], endometrial tissue throughout the menstrual cycle [28], or CH/LC [29-32]. Although expression of CD105 along hepatic sinusoids (CD105(HS)) in CH/LC has been reported [29-32], a correlation between CD10 and CD105 expression has not been examined so far. We previously reported changes in expression of CD10 and CD105 in the hepatic tissue around tumors including HCC and metastatic carcinoma [33]. In the present study, we analyzed CH/LC in the same way as in the previous report.

2. Materials and methods

2.1. Tissue samples

Fifty-two cases of surgically resected liver specimens were retrieved from a pathological database file at Suwa Red Cross Hospital (Suwa, Japan). The cases showing fatty metamorphosis or treated before operation by trans-arterial embolization or radiofrequency ablation for tumors were excluded. The resected livers had HCC with CH or LC (40 cases, consisted of 26 cases of type C, 4 of type B and 10 of non-B/non-C type), metastatic carcinoma (11 cases; 8 for colorectal origin and 1 for breast, stomach and Vater's papilla origin in each) and biliary cystadenoma (1 case). None of the cases of metastatic carcinoma and cystadenoma showed features of CH/LC clinically or histopathologically. All specimens had been fixed in 10% phosphate-buffered formalin immediately after resection and embedded in paraffin. In each case, representative paraffin blocks of the background hepatic tissue of tumors were selected and serial tissue sections were subjected to Azan-Mallory and immunohistochemical stainings. The sections contained part of the tumor nodule in 46 cases.

2.2. Evaluation of chronic hepatitis/cirrhosis

The inflammatory activity (A) and fibrosis stage (F) of CH/LC were evaluated by histological observation of sections with hematoxylin-eosin and Azan-Mallory stainings according to the new Inuyama Classification of Japan [34], which well corresponds to the International Classification of chronic hepatitis [35]. The inflammatory activity was classified as A0 (no inflammation or necrosis), A1 (mild), A2 (moderate) and A3 (severe), and the fibrosis stage as F0 (no fibrosis), F1 (fibrous extension of Glisson's sheathes), F2 (bridging fibrosis), F3 (distorted bridging fibrosis or pre-cirrhotic state) and F4 (LC). The background hepatic tissues apart

enough from the tumor nodule in the cases of metastatic carcinoma or cystadenoma were used as controls.

2.3. Immunohistochemistry and evaluation of staining

The tissue sections were stained immunohistochemically using the streptavidin-biotin-peroxidase method. Endogenous peroxidase activity in the sections was eliminated in 0.3% hydrogen peroxide in methanol for 30 min. Thereafter, the sections were treated with 0.2% trypsin for 40min. at 37 degrees C (for CD105) or were submerged in 0.01mol/l citrate buffer (pH6.0) and boiled for 15 minutes in a microwave oven (for CD10) to retrieve the antigenecity [36]. After cooling to room temperature, these sections were incubated with monoclonal antibodies against CD10 (clone 56C6, Medical & Biological Laboratories, Nagoya, Japan, 1:50 dilution) and CD105 (clone SN6h, Dako, Glostrup, Denmark, 1:5 dilution), followed by biotinylated antibody against mouse immunoglobulin (Dako, 1:50 dilution), and finally reacted with peroxidase-conjugated streptavidin (Dako). The labeled peroxidase was visualized by 3,3′-diaminobenzidine-hydrogen peroxide method and counterstained by hematoxylin. Negative control sections for immunostaining were treated with phosphate-buffered saline instead of the primary antibodies and were confirmed to be unstained.

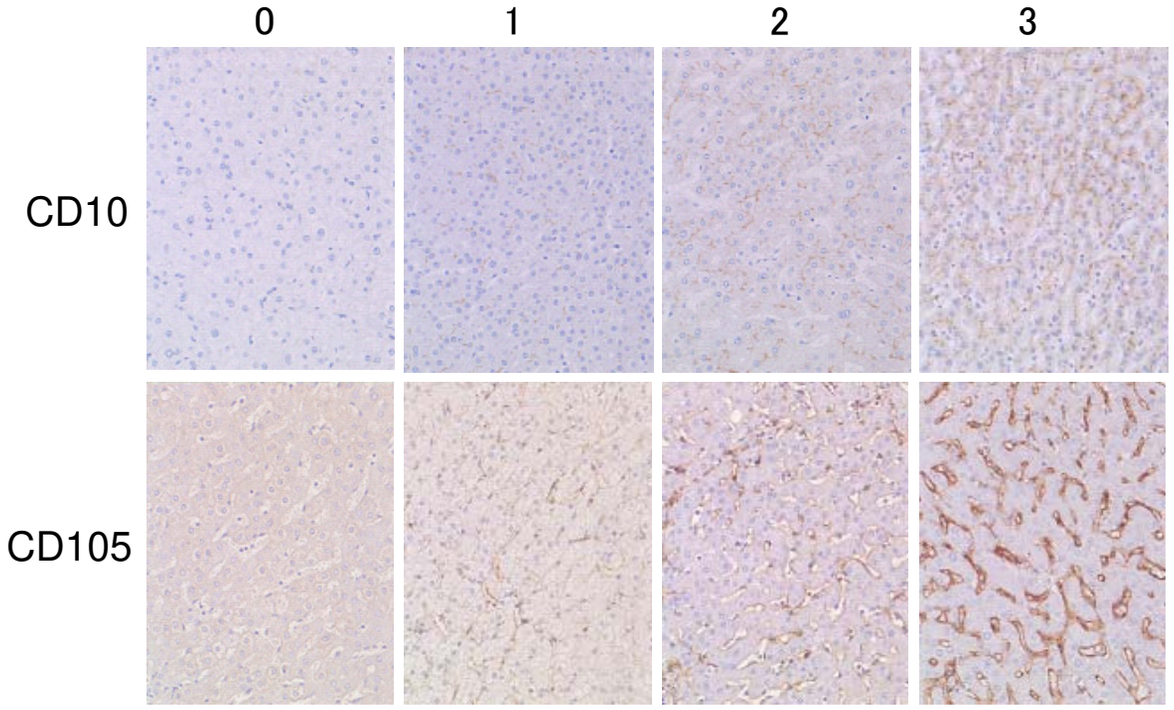

Figure 1. Representative features of CD10 score in bile canaliculi and CD105 score along hepativ sinusoids in lobular areas.

A scoring system was introduced for evaluation of expression of CD10 or CD105 in the background hepatic tissue, apart more than 5mm. from the tumor nodule. The intensity of CD10- or CD105-reactivity in the peri-portal and lobular areas were evaluated separately as 0

(no staining), 1 (weak), 2 (moderate) and 3 (intense) (Figure 1), and the sum of peri-portal and lobular scores was defined as CD10 score or CD105 score respectively. The immunoreactivity for CD10 in HCC was also evaluated as 0, 1, 2 and 3, and was defined as CD10-T. Statistical analyses of the scores were performed mutually or in relation to the severity of CH/LC by Mann-Whitney's test using Statmate III software (Atoms, Tokyo, Japan). Spearman's rank correlation coefficient between CD10 score and CD105 score was calculated by Statmate III.

3. Results

3.1. Control hepatic tissues

Out of 12 cases of metastatic carcinoma or biliary cystadenoma, the background hepatic tissues in 10 cases were evaluated as A0/F0 and those in the remaining 2 as A1/F0 and A0/F1 respectively. CD10(BC) expression was not uniform, while a difference of immunoreactivity was not conspicuous between peri-portal and lobular areas. CD10 score was more than 4 (moderate to intense staining) in 7 out of these 12 cases (Figure 2A, Table 1), including a case of A0/F1.

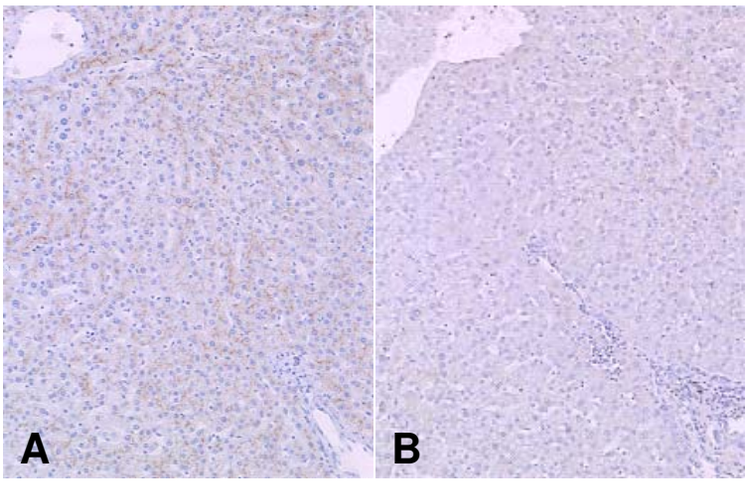

Figure 2. Expression of CD10 (A) and CD 105 (B) in the control hepatic tissue (serial sections). Bile canaliculi are moderately to intensely immunoreactive for CD10, whereas CD105 is not expressed in any regions.

As for CD105, 5 cases (including a case of A0/F1) showed no expression in any region of the background hepatic tissues (Figure 2B, Table 2). The other 7 cases did not reveal CD105(HS)-immunoreactivity in peri-portal areas but showed focal/weak expression in the lobular areas.

3.2. Chronic hepatitis/cirrhosis

CD10(BC) expression in the cases with CH/LC was not uniform, as in the control hepatic tissue, while CD10 scores were, in general, significantly lower than those in the control hepatic tissue (Figure 3A, Table 1). However, CD10(BC)-immunoreactivity was well preserved in the areas that the hepatocyte plates were regularly arranged with distinct trabecular pattern in some

cases (Figure 3B). In relation to inflammatory activity or fibrosis, a significant difference of CD10 score was demonstrated between the cases of A0 and A1-3 or between those of F0 and F1-4, but, among each group, only between A0 and A1 or F1-2 and F3 (Figures 4 B and 4E, Table 1).

CD105-positive hepatic sinusoids also revealed uneven distribution in the hepatic lobules. Although CD105 score was variable from case to case (Figures 4 C and 4F), the scores in CH/LC were significantly higher than those in the control hepatic tissue (Table 2). They also showed a tendency to be high in the cases of prominent inflammatory activity but not in those of advanced fibrosis stage (Table 2).

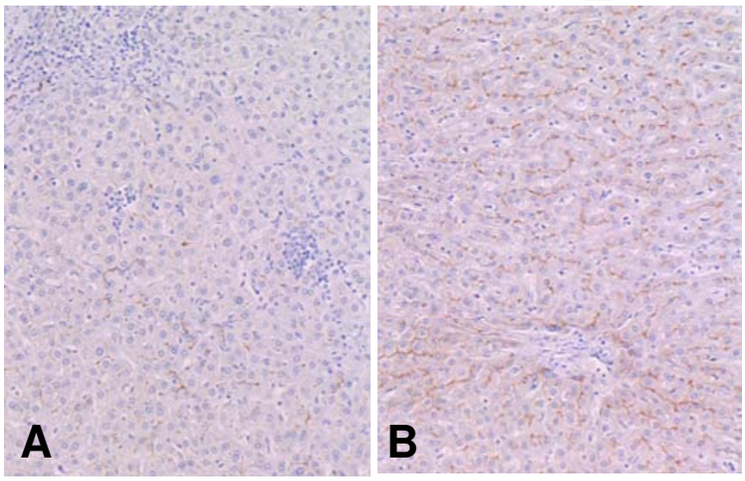

Figure 3. CD10 expression in a case of chronic hepatitis (A2/F2). (A) Decreased expression in the area of moderate inflammatory acitivity. (B) Intense expression in the area of mild inflammatory activity and well-preserved trabecular arrangement of hepatocyte plates.

CD10 score	0	1	2	3	4	5	6	
Control (12)		1	1	3	2	2	3	control vs. CH/LC: $p<0.05$
CH/LC (40)	12	6	8	7	4	3		
A0 (11)		1		3	2	2	3	
A1 (20)	6	1	6	4	1	2		A0 vs. A1-3: $p<0.001$
A2 (18)	5	4	2	3	3	1		A0 vs. A1: $p<0.01$
A3 (3)	1	1	1					A1 vs. A2: NS
F0 (13)		1	2	4	2	2	2	
F1 (4)				2		1	1	F0 vs. F1-4: $p<0.005$
F2 (5)			2		2	1		F1 vs. F1-2: NS
F3 (11)	5	2	3	1				F1-2 vs. F3: $p<0.001$
F4 (19)	7	4	2	3	2	1		F3 vs. F4: NS

NS: not significant

( ): number of cases

Table 1. CD10 score in bile canaliculi in control liver and CH/LC in relation to inflammatory acitivity and degree of fibrosis

CD105 score	0	1	2	3	4	5	
control (12)	5	5	2				control vs. CH/LC: p＜0.001
CH/LC (40)	4	9	11	10	4	2	
A0 (11)	5	4	2				
A1 (20)	3	8	5	3	1		A0 vs. A1-3: p＜0.01
A2 (18)	1	2	6	5	3	1	A0 vs. A1: p＜0.05
A3 (3)				2		1	A1 vs. A2: p＜0.05
F0 (13)	4	6	3				
F1 (4)	2	1			1		F0 vs. F1-4: p＜0.005
F2 (5)	2		1	1		1	F0 vs. F1-2: NS
F3 (11)	1	4	4		1	1	F1-2 vs. F3: NS
F4 (19)		3	5	9	2		F3 vs. F4: NS
( ): number of cases							NS: not significant

Table 2. CD105 score along hepatic sinusoid in control liver and CH/LC in relation to inflammatory activity and degree of fibrosis

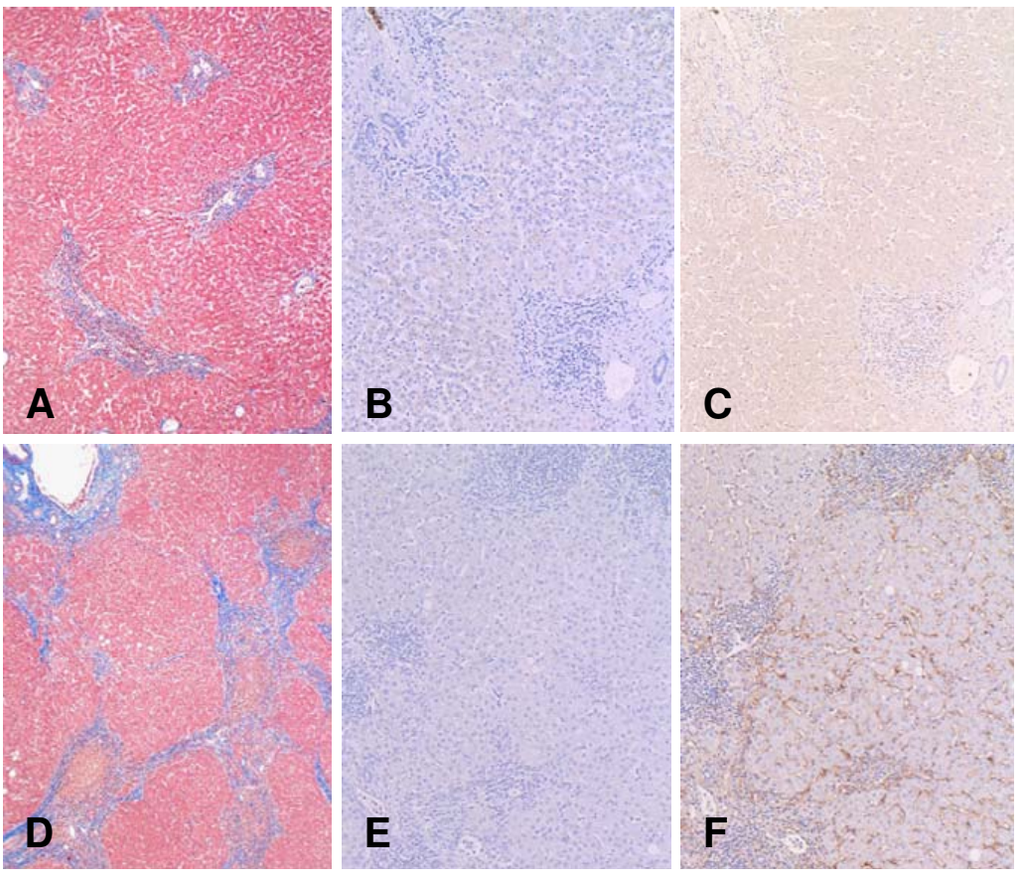

Figure 4. Representative cases of chronic hepatitis (CH): (A)-(C) CH (A1/F1), (D)-(F) CH (A2/F3). (A)(D) Azan-Mallory staining, (B) CD10 score 3, (C) CD105 score 0 (serial section of (B)), (E) CD10 score 0, (F) CD105 score 4 (serial section of (E)).

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Figure 5 shows a correlation between CD10 score and CD105 score in all cases examined (including CL/LC and control hepatic tissues). There was a significant inverse correlation and Spearman's rank correlation coefficient was -0.533.

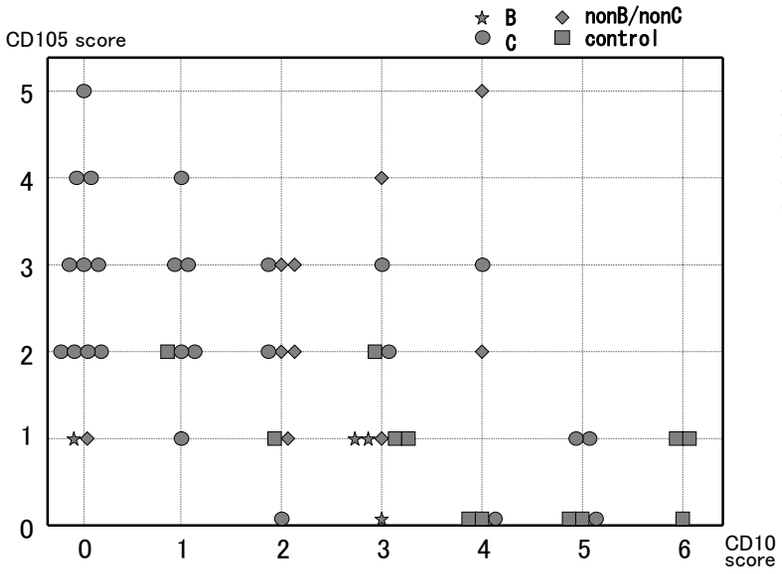

Figure 5. Inverse correlation of CD10 score and CD105 score (r_s=-0.533).

3.3. CD10 expression in HCC and background hepatic tissue

CD10 expression was observed, at least in part of HCC tissue, in 20 (58.8%) out of 34 cases examined (Figure 6). Although the immunoreactivity was variable, the cases with CD10-positive tumor tissues showed significantly higher CD10 score in the background hepatic tissue than those with no intra-tumoral CD10 expression (Table 3).

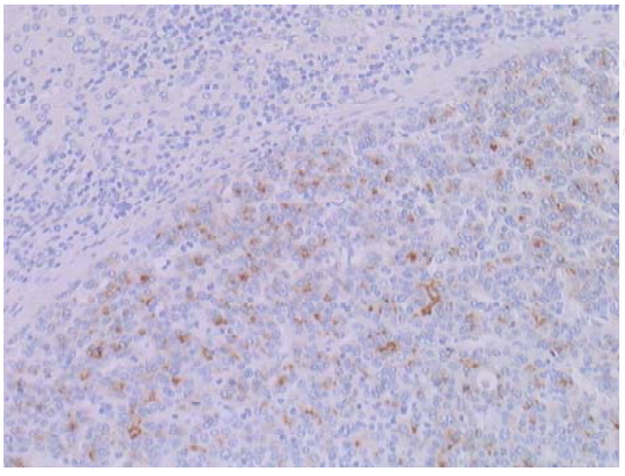

Figure 6. Expression of CD10 in hepatocellular carcinoma.

CD10 score in background liver tissue	0	1	2	3	4	5	
CD10 in HCC (−)	6	2	2	2	1	1	$p<0.05$
(+)	4	1	6	5	2	2	

Table 3. CD10 expression in hepatocellular carcinoma and CD10 score in background liver tissue

4. Discussion

In normal human liver, CD10 is detected in the bile canaliculi and interlobular bile ducts [6,7]. The present study indicated a decrease of CD10(BC) expression in CH/LC, a similar finding to what has been recently reported [15]. The distribution of CD10-immunoreactivity was not uniform, while the regularly arranged hepatocyte plates with distinct sinusoids maintained CD10-positive bile canaliculi. These observations may indicate, in CH/LC, a loss of differentiation and/or a functional impairment of bile canaliculi due to persistent hepatocyte injury. According to Shousha *et al.* [15], the loss of CD10(BC) reactivity was significantly correlated with fibrosis stage, but not with necro-inflammatory grade. In our study, however, a significant difference of CD10(BC) expression was demonstrated according to the presence or absence of inflammatory activity or fibrosis, but not necessarily between the groups of different grades of them. These results suggest that a decrease of CD10(BC) expression does not simply depend on the grade of inflammatory activity or fibrosis. Concerning other factors in relation to the severity of CH/LC, Shousha *et al.* reported a correlation of decreased CD10(BC) expression with abnormalities in liver function tests in CH/LC [15]. Meanwhile, our preliminary examination on alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (gamma-GTP) showed that there was no distinct correlation between CD10 score and any data described above (data not shown). Further investigation on the mechanism of change in expression of CD10(BC) is warranted.

CD10 has also been used as a reliable marker for pathological diagnosis of HCC. The positive rate (58.8%) of CD10 expression in HCC in the present study was consistent with the previous reports (about 52-68%) [6,7,13,14]. In addition, CD10 in HCC was significantly correlated with the CD10 score of background hepatic tissue, although there was no significant correlation between CD10-T in HCC and CD score *in the lobular areas* in the previous report [33]. The present study suggests a phenotypic similarity of neoplastic and non-neoplastic hepatic tissue and also indicates the importance of evaluation of CD10 score in the all (peri-portal and lobular) areas.

The current study disclosed a significant inverse correlation between CD10 score and CD105 score in CH/LC. It has been reported that CD105 is not or minimally expressed in sinusoidal endothelial cells in normal human liver and is up-regulated in various types of chronic diseases, such as viral CH [29-32], autoimmune hepatitis [37], primary biliary cirrhosis [37,38],

alcoholic liver disease [39,40] and various benign nodular lesions [27]. Recently, it has been reported that increased CD105(HS) expression is significantly associated with progressive hepatic fibrosis in chronic hepatitis C virus infection [31]. The present study also showed up-regulation of CD105(HS)-immunoreactivity in CH/LC, indicating neoangiogenesis of hepatic sinusoids [30], and it was significantly correlated with the degree of inflammatory activity but not with the degree of fibrosis. The discrepancy between the two reports may reflect differences of methods of grading and scoring system. As for the type of hepatitis virus, CD105 score in CH/LC caused by hepatitis B virus was 0 or 1 in all cases in the present study. A correlation between CD105 score and type of hepatitis virus, however, could not be clarified because the number of cases of type B CH/LC was too small. Further cases of hepatitis B virus-positive CH/LC should be analyzed, although its infectious rate has been recently decreased due to vaccination [41].

In conclusion, the present study indicates simultaneous down-regulation of CD10(BC) and up-regulation of CD105(HS) in CH/LC. Although the phenotypic changes of bile canaliculi and hepatic sinusoids may be caused by separate mechanisms, they seem to represent different aspects induced by a common event, i.e., persistent hepatic injury.

5. Summary

The present study was undertaken in order to examine expression of CD10 in bile canaliculi (CD10(BC)) in relation to that of CD105 (endoglin) along hepatic sinusoids (CD105(HS)) in chronic hepatitis and liver cirrhosis (CH/LC). Fifty-two cases of resected liver, bearing hepatocellular carcinoma (HCC), metastatic carcinoma or biliary cystadenoma, were immunostained for CD10 and CD105. The immunoreactivity for CD10(BC) and CD105(HS) in the background hepatic tissue of tumors was scored separately. In the background hepatic tissue of metastatic carcinoma or cystadenoma (as controls), CD10(BC) was moderately or markedly expressed in more than half of the cases, whereas CD105(HS) was not or minimally positive. Compared with the controls, CH/LC cases significantly showed a decrease of CD10 score and an increase of CD105 score, the latter indicating neoangiogenesis, with inverse correlation (r_s = -0.533). The down-regulation of CD10 was not necessarily correlated with inflammatory activity or degree of fibrosis, whereas the up-regulation of CD105 was significantly correlated with inflammatory activity. These results indicate that the expression pattern of CD10(BC) and CD105(HS) changed simultaneously in CH/LC by persistent hepatic injury, although the mechanism of change of these markers may be different.

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