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RAPID COMMUNICATION

Ductular proliferation in liver tissues with severe chronic hepatitis B: An immunohistochemical study

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Abstract

AIM: To clarify the pathogenesis of ductular proliferation and its possible association with oval cell activation and hepatocyte regeneration.

METHODS: Immunohistochemical staining and image analysis of the ductular structures in the liver tissues from 11 patients with severe chronic hepatitis B and 2 healthy individuals were performed. The liver specimens were sectioned serially, and then cytokeratin 8 (CK8), CK19, OV6, proliferating cell nuclear antigens (PCNA), glutathione-S-transferase (GST), α-fetal protein (AFP) and albumin were stained immunohistochemically.

RESULTS: Typical and atypical types of ductular proliferation were observed in the portal tracts of the liver tissues in all 11 patients. The proliferating ductular cells were positive for CK8, CK19, OV6 and PCNA staining. Some atypical ductular cells displayed the morphological and immunohistochemical characteristics of hepatic oval cells. Some small hepatocyte-like cells were between hepatic oval cells and mature hepatocytes morphometrically and immunohistochemically.

CONCLUSION: The proliferating ductules in the liver of patients with severe chronic liver disease may have different origins. Some atypical ductular cells are actually activated hepatic oval cells. Atypical ductular proliferation is related to hepatocyte regeneration and small hepatocyte-like cells may be intermediate transient cells between hepatic oval cells and mature hepatocytes.

Key words: Ductular proliferation; Chronic hepatitis B; Hepatocyte regeneration

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INTRODUCTION

Ductular proliferation is often used to describe the appearance of biliary epithelial cells in the portal tracts of diseased livers. The proliferating bile ductules are heterogeneous and are histologically classifiable into typical and atypical types[1]. Atypical and typical ductules have been reported in long-standing biliary diseases such as primary biliary cirrhosis, primary sclerosing cholangitis, extrahepatic biliary obstruction, etc[2-5]. Bile ductular proliferation is also the most commonly observed finding in patients with chronic hepatitis C[6,7]. According to our experience, however, such proliferating ductules are also frequently seen in patients with severe or end-stage chronic liver disease induced by hepatitis B virus (HBV). It is still not clear why these ductular structures are often increased in the liver tissue of such patients. One possibility is that the proliferating ductular cells are human counterparts of rat oval cells or these proliferating ductules may be engaged in hepatocyte regeneration[8-10].

To explore the pathogenesis of ductular proliferation and its possible association with hepatic oval cell activation in patients with severe chronic hepatitis B, we performed immunohistochemical staining and image analysis of the ductular structures in the liver tissues of such patients.

MATERIALS AND METHODS

Liver tissues

A total of 11 liver specimens were obtained from patients (9 male and 2 female) with severe chronic hepatitis B. The average age of the patients was 39.2 years, ranging from 22 to 54. The total serum bilirubin of all patients exceeded 171 μmol/L and the plasma thrombinogen activity of the patients decreased to 40% or less. Among the 11 patients, 10 were infected with HBV and 1 was superinfected with HBV and hepatitis D virus. All of the liver tissue specimens were obtained by needle puncture or by autopsy. For control purposes, archival normal liver tissue was obtained from 2 subjects who had no abnormal liver pathology and

Table 1 Average optical density of typical and atypical ductular cells for CK8, CK19 and OV6 staining

Cell Types	Fields	CK8	CK19	OV6
Typical	8	0.402 ± 0.083	0.902 ± 0.355	0.324 ± 0.121
Atypical	9	0.998 ± 0.238[b]	0.371 ± 0.105[b]	0.752 ± 0.210

[b]$P < 0.001$ *vs* typical

Table 2 Morphometric parameters of hepatic oval cells, hepatocyte-like cells and hepatocytes

Cell types	Cell numbers	At (μm^2)	D_{max} (μm)	D_{max}/D_{min} ratio
Hepatic oval cells	97	19.5 ± 5.9	12.4 ± 7.4	1.8 ± 0.5
Hepatocyte-like cells	42	32.1 ± 6.3[b]	18.9 ± 7.8[b]	1.2 ± 0.1
Hepatocytes	114	41.5 ± 2.3	24.7 ± 5.1	1.1 ± 0.1

[b]$P < 0.001$ *vs* hepatic oval cells and hepatocytes

no biochemical or serological evidence of liver disease. All 10 specimens were fixed in 10% neutral-buffer formalin and embedded in paraffin.

Histology and immunohistochemistry

Serial 4 μm thick sections were prepared and the sections were deparaffinized in xylene and rehydrated through graded alcohol. Part of the sections were stained with hematoxylin and eosin for histological diagnosis. The remaining sections were processed for immunohistochemical staining and a three-step indirect immunoperoxidase procedure was used. The sections were digested with protease (Sigma Chemical Co., St. Louis, MO) for 4 min at 37℃ or boiled in 10 mmol/L citrate buffer, pH6.0, in a microwave for two treatments of 2 min. Endogenous peroxidases were inactivated by immersing the sections in 3% hydrogen peroxide for 10 min. Sections to be used were incubated for 10 min with normal goat serum in Tris-buffered saline to block nonspecific binding. The sections were subsequently incubated overnight at 4℃ with the relevant antibodies. The following day, the sections were incubated with biotinylated anti-mouse IgG (Dako A/S, Glostrup, Denmark) for 45 min at 37℃. The sections were then incubated with peroxidase-conjugated streptavidin (Dako A/S, Glostrup, Denmark) for 45 min at 37℃. The chromogenic reaction was developed with diaminobenzidine and all of the sections were counterstained with hematoxylin. The monoclonal antibodies used were mouse-anti-human cytokeratin 8 (CK8) (Dako A/S, Glostrup, Denmark), CK19 (Serotec Ltd, UK), mouse-anti-human proliferating cell nuclear antigen (PCNA)(Zymed Laboratories, Inc., CA), mouse-anti-human α-fetal protein (AFP) (Zymed Laboratories, Inc., CA), mouse-anti-human glutathione-S-transferase (GST), mouse-anti-human albumin (Zymed Laboratories, Inc., CA) and mouse-anti-rat OV6 (a kind donation from Dr. D. Hixson, Brown University, USA).

Image analysis

Cell image analysis was performed on the liver tissue sections by Tiger® cell image analysis system (Center of Industrial Computer Tomography, Chongqing University, Chongqing, China) to determine total cell area (At), maximum diameter (D_{max}), minimum diameter (D_{min}), D_{max}/D_{min} ratio and average optical density of proliferating ductular structures.

Statistical analysis

Data were analyzed using the t test. $P<0.05$ was considered statistically significant.

RESULTS

Histopathological findings

Massive/submassive hepatocyte necrosis, lymphocyte infiltration, fibrous tissue hyperplasia and cholestasis were observed apparently in the liver tissues of all 11 patients. Hepatocyte proliferation foci and pseudolobules were seen in 6 and 4 patients respectively. In the portal tracts of all the liver specimens from the patients, obviously increased bile ductules were seen, some of which invaded into parenchyma. However, only 1 or 2 bile ductules were observed in the portal tracts of normal liver tissues. The proliferating ductules were also classifiable into typical and atypical types, but cell image analysis showed that the ductular cells from typical and atypical ductules had no significant difference in At, D_{max} and D_{max}/D_{min} ratio.

Immunohistochemical findings

Both types of proliferating ductular cells of patient group were positive for CK8, CK19, OV6 and PCNA staining. However, typical proliferating ductular cells showed strong staining for CK19 and weak staining for CK8 and OV6. In contrast, some atypical proliferating ductular cells showed strongly positive for CK8 and OV6 staining and weakly positive for CK19 staining. There was no significant difference between typical and atypical ductular cells for PCNA staining. In the normal liver tissue taken as control, ductular cells were positive only for CK19 staining. Image analysis demonstrated that typical and atypical ductular cells had significant difference in the intensity and extent of staining (Table 1).

Atypical ductular cells and hepatic oval cells

Morphometrically, the proliferating ductular cells were similar to hepatic oval cells, which were characterized by ovoid nuclei and scanty basophilic cytoplasm. Some atypical ductular cells expressed phenotypes of both ductular cells and fetal hepatocytes, including CK8, CK19, OV6, AFP and GST. These cells were consistent with hepatic oval cells morphologically and immunohistochemically.

Ductular proliferation and liver regeneration

The foci of hepatocyte proliferation were observed in the liver tissues of 6 patients. The regenerating hepatocytes were characterized by different size of cells, anisokaryosis and basophilic cytoplasm. Some hepatocyte-like cells surrounding the proliferative foci were between hepatic oval cells and mature hepatocytes in cell size and morphology (Table 2), and were positive for AFP, GST, OV6 and albumin, demonstrating the features of both hepatocytes and biliary epithelial cells.

DISCUSSION

Bile ductules connect bile canaliculi of periportal hepatocytes with interlobular bile ducts in portal tracts. The bile canaliculi, which form a complicated polyponal network with many anastomotic interconnections, drain into a relatively few bile ductules in periportal area. These bile ductules adjacent to bile canaliculi are the commencement of the biliary tree, and they are composed of ductular cells and hepatocytes. Some may show slight luminal dilatation where several bile canaliculi converge. In normal livers, only a few bile ductules are recognizable in a portal tract, while in various chronic hepatobiliary diseases these ductular structures are often increased. The pathogenesis and significance of this kind of ductular proliferation differ from disease to disease, but generally speaking, typical type of proliferating ductules originate from preexisting ductules and atypical type of proliferating ductules have more complicated origins and may be related to the activation of hepatic stem cells and the transformation of hepatocytes[11].

In ordinary circumstances, liver regeneration is usually achieved by the entry of normal, proliferatively quiescent, differentiated hepatocytes into the cell cycles, but, when hepatocyte regeneration is defective, bile ductular cells can migrate outwards from the portal tracts and then differentiate into hepatocytes. These biliary cells are called hepatic oval cells or oval cells, and their emergence when hepatocyte regeneration is impaired suggests they are the progeny of hepatic stem cells. End-stage chronic liver disease often develops from advanced chronic liver disease and is characterized by massive/submassive hepatocyte necrosis and severe liver failure. The mortality of severe chronic hepatitis B is very high. Our data showed that 72.5% (306/422) of such patients would die of the disease[12]. Theoretically, hepatic stem cells might be activated and proliferated in the liver of those patients. On one hand, massive/submassive necrosis of hepatocytes leads to a sharp decrease in the mass of hepatocytes, and thus the liver has a demand of regeneration. On the other hand, various toxins resulting from hepatocyte necrosis inhibit the proliferation and regeneration of residual hepatocytes. These conditions will activate hepatic stem cells and induce them to proliferate and differentiate toward hepatocytes. However, the above speculation has yet to be confirmed. Investigation of the nature of ductular proliferation will help to understand the mechanism of hepatocytes regeneration in the liver tissues of chronic hepatitis B patients.

Our results showed that two types of ductular proliferation, typical and atypical, existed in the liver of patients with severe chronic hepatitis B, and the phenotypes of the two types of ductular cells differed in some ways. The former were characterized by well-formed lumina and distinct cell borders and the latter characteristically had vague or no visible lumina and rather indistinct cell border. The typical ductular cells and ductules in normal liver were strongly positive for CK19 staining, suggesting that typical ductules originated from preexisting ductules. Some atypical ductular cells displayed the phenotypes of both biliary duct cells and hepatocytes and had the morphometric features of hepatic oval cells, suggesting that at least part of the atypical ductular cells originated from hepatic stem cells. It implied that some atypical ductular cells were actually activated hepatic oval cells. Our results also demonstrated that hepatocyte regeneration in the liver of patients with severe chronic liver disease was related to ductular proliferation. Firstly, some atypical ductular cells were actually activated hepatic oval cells, which could differentiate into hepatocytes ultimately. Secondly, there was histopathological and immunochemical evidence of hepatocyte regeneration in the liver of the patients. And thirdly, some regenerating small hepatocyte-like cells were between hepatic oval cells and mature hepatocytes morphometrically and immunohistochemically, suggesting that these cells were differentiated from hepatic oval cells and were the transient cell type from hepatic oval cells to mature hepatocytes. These findings are consistent with those of *Tan et al*[13]. It is believed that liver stem cells are a potential source of hepatocytes[14-16]. Although hepatic stem cells are activated and can differentiate into hepatic oval cells and transient hepatocyte-like cells ultimately, it is not clear why the diseased liver cannot be regenerated completely. We speculate that the toxins released from hepatocyte necrosis inhibit not only the proliferation and regeneration of residual hepatocytes but also the differentiation and evolution of hepatic oval cells toward hepatocytes.

In conclusion, the proliferating ductules in the liver of patients with severe chronic hepatitis B may have different origins. Some atypical ductular cells are actually activated hepatic oval cells. Atypical ductular proliferation is related to hepatocyte regeneration and small hepatocyte-like cells may be intermediate transient cells between hepatic oval cells and mature hepatocytes. However, why the diseased liver cannot be regenerated completely after hepatic stem cell activation remains to be investigated further.

REFERENCES

1 **Bisgaard HC,** Holmskov U, Santoni-Rugiu E, Nagy P, Nielsen O, Ott P, Hage E, Dalhoff K, Rasmussen LJ, Tygstrup N. Heterogeneity of ductular reactions in adult rat and human liver revealed by novel expression of deleted in malignant brain tumor 1. *Am J Pathol* 2002; **161:** 1187-1198

2 **Roskams TA,** Theise ND, Balabaud C, Bhagat G, Bhathal PS, Bioulac-Sage P, Brunt EM, Crawford JM, Crosby HA, Desmet V, Finegold MJ, Geller SA, Gouw AS, Hytiroglou P, Knisely AS, Kojiro M, Lefkowitch JH, Nakanuma Y, Olynyk JK, Park YN, Portmann B, Saxena R, Scheuer PJ, Strain AJ, Thung SN, Wanless IR, West AB. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology* 2004; **39:** 1739-1745

3 **Saxena R,** Hytiroglou P, Thung SN, Theise ND. Destruction of canals of Hering in primary biliary cirrhosis. *Hum Pathol* 2002; **33:** 983-988

4 **Quaglia A,** Tibballs J, Grasso A, Prasad N, Nozza P, Davies SE, Burroughs AK, Watkinson A, Dhillon AP. Focal nodular hyperplasia-like areas in cirrhosis. *Histopathology* 2003; **42:** 14-21

5 **Azar G,** Beneck D, Lane B, Markowitz J, Daum F, Kahn E. Atypical morphologic presentation of biliary atresia and value of serial liver biopsies. *J Pediatr Gastroenterol Nutr* 2002; **34:** 212-215

6 **Clouston AD,** Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonsson JR. Fibrosis correlates with a ductular reaction

in hepatitis C: roles of impaired replication, progenitor cells and steatosis. *Hepatology* 2005; **41:** 809-818
7 **Sagnelli E,** Pasquale G, Coppola N, Marrocco C, Scarano F, Imparato M, Sagnelli C, Scolastico C, Piccinino F. Liver histology in patients with HBsAg negative anti-HBc and anti-HCV positive chronic hepatitis. *J Med Virol* 2005; **75:** 222-226
8 **Saxena R,** Theise N. Canals of Hering: recent insights and current knowledge. *Semin Liver Dis* 2004; **24:** 43-48
9 **Alison MR,** Vig P, Russo F, Bigger BW, Amofah E, Themis M, Forbes S. Hepatic stem cells: from inside and outside the liver? *Cell Prolif* 2004; **37:** 1-21
10 **Roskams TA,** Libbrecht L, Desmet VJ. Progenitor cells in diseased human liver. *Semin Liver Dis* 2003; **23:** 385-396
11 **Jensen CH,** Jauho EI, Santoni-Rugiu E, Holmskov U, Teisner B, Tygstrup N, Bisgaard HC. Transit-amplifying ductular (oval) cells and their hepatocytic progeny are characterized by a novel and distinctive expression of delta-like protein/preadipocyte factor 1/fetal antigen 1. *Am J Pathol* 2004; **164:** 1347-1359
12 **Wang Y,** Chen Y, Gu C, Jiang L and Xiang D. Reevaluation of the nomenclature and diagnostic criteria in 477 patients with severe hepatitis. *Zhonghua Ganzangbing Zazhi* 2000; **8:** 261-263
13 **Tan J,** Hytiroglou P, Wieczorek R, Park YN, Thung SN, Arias B, Theise ND. Immunohistochemical evidence for hepatic progenitor cells in liver diseases. *Liver* 2002; **22:** 365-373
14 **Quesenberry PJ,** Dooner G, Colvin G, Abedi M. Stem cell biology and the plasticity polemic. *Exp Hematol* 2005; **33:** 389-394
15 **Burke ZD,** Tosh D. Therapeutic potential of transdifferentiated cells. *Clin Sci (Lond)* 2005; **108:** 309-321
16 **Parenteau NL,** Rosenberg L, Hardin-Young J. The engineering of tissues using progenitor cells. *Curr Top Dev Biol* 2004; **64:** 101-139

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