Possible stem cell origin of human cholangiocarcinoma

Chao Liu, Jie Wang, Qing-Jia Ou

Chao Liu, Jie Wang, Qing-Jia Ou, Department of General Surgery, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510120, Guangdong Province, China

Corresponding to: Dr. Chao Liu, Department of General Surgery and Transplantation, University Hospital Essen, Hufelandstr. 55, Essen D-45122, Germany. mliuchao@hotmail.com
Received: 2004-02-28 Accepted: 2004-04-29

Abstract

AIM: To investigate the expression of CD34 and c-kit (receptor of stem cell factor) in cholangiocarcinoma.

METHODS: Fifteen cases of intrahepatic cholangiocarcinoma and 17 cases of extrahepatic cholangiocarcinoma were studied in this experiment. Using Envision detection system, paraffin-embedded sections of the resected cholangiocarcinoma tissue were stained with antibodies against CD34 and c-kit, respectively. The sections were counterstained with hematoxylin, and the results were examined under light microscope. Normal tonsil and mammary tissues were used as positive controls for CD34 and c-kit, respectively.

RESULTS: CD34 was positive in all sections, but only in capillary endothelial cells of tumor tissue. No cholangiocarcinoma cells were positive for CD34. In one case of extrahepatic cholangiocarcinoma, a few tumor cells (about 5%) were immunoreactive with c-kit.

CONCLUSION: CD34 or c-kit positive cells in liver tissue may represent liver stem cells, as they can differentiate into mature biliary cells in vitro. The expression of c-kit by some cholangiocarcinoma cells suggests that cholangiocarcinoma might originate from liver stem cells. However, other mechanisms of hepatocarcinogenesis, such as de-differentiation of mature cholangiocytes, may also exist.

Liu C, Wang J, Ou QJ. Possible stem cell origin of human cholangiocarcinoma. World J Gastroenterol 2004; 10(22): 3374-3376
http://www.wjgnet.com/1007-9327/10/3374.asp

INTRODUCTION

Two theories are available to explain the process of hepatocarcinogenesis, one is de-differentiation of mature liver cells (hepatocytes and cholangiocytes), the other is maturation arrest of liver stem cells[1]. In normal liver, putative liver stem cells may exist at terminal bile ductules (canal of Hering) and periductular area[2,3]. In rodent animals, when damage and loss of hepatocytes and/or cholangiocytes are combined with impaired regeneration of the mature cells, liver stem cells may be activated. They proliferate and differentiate towards both hepatic and biliary lineages[4-7]. Activation of liver stem cells has been observed in various human liver diseases, such as acute liver necrosis[8], hemochromatosis[9], chronic cholestatic diseases[10], alcoholic liver diseases[9] and chronic viral hepatitis[9,11,12]. In human liver focal nodular hyperplasia[13], hepatic adenoma[14], hepatocellular carcinoma[15] and hepatoblastoma[16], some tumor cells have also been detected to express the specific markers of liver stem cells, indicating their possible stem cell origin. In animals, cholangiocarcinoma can also originate from liver stem cells[17].

CD34 and c-kit are two hematopoietic markers, but in periductular area and occasionally within bile ducts, CD34 and c-kit positive area was also found[18]. CD34 or c-kit positive cells in human liver can be isolated with immunomagnetic separation techniques, and these isolated cells are able to differentiate into biliary epithelial cells in vitro[19]. Thus, CD34 and c-kit positive cells in human liver may represent liver stem cells. In this study, the expression of CD34 and c-kit in human cholangiocarcinoma was investigated.

MATERIALS AND METHODS

Specimens

Paraffin-embedded specimens from 32 cases of resected cholangiocarcinoma at Sun Yat-Sen Memorial Hospital were studied in this experiment. They included 18 male and 14 female patients, ranging from 24 to 80 years old (mean and medium 64 years old). Fifteen cases had the tumor located in intrahepatic bile duct (IBD), 4 cases in common hepatic bile duct (CHBD) and 13 cases in common bile duct (CBD). Some clinical characteristics of the patients are summarized in Table 1.

Immunohistochemistry

Each paraffin-embedded specimen was cut consecutively into 6 sections. Three sections of CD34 and 3 sections of c-kit were stained with Envision detection system (DAKO, Denmark). CD34 retrieval was performed by heating the sections in 10 mmol/L citrate buffer (pH 6.0). In brief, the tissue sections were incubated with peroxidase blocking reagent (DAKO) for 5 min, incubated with CD34 (monoclonal mouse anti-human, IgG1, kappa, ready to use; DAKO) for 10 min or c-kit (polyclonal rabbit anti-human, 50; DAKO) for 30 min at room temperature. Then, the sections were incubated with peroxidase labelled polymer conjugated to goat anti-rabbit or goat anti-mouse immunoglobulin for 30 min at room temperature, incubated with diaminobenzidine (DAB) chromogen for 5 min, counterstained with hematoxylin and mounted with coverslip. Between each of these steps, the sections were rinsed gently with Tris-HCl buffer. Normal human tonsil and mammary tissues were used as positive controls for CD34 and c-kit, respectively. Negative control was performed at the same conditions by omitting incubation with the first antibody. The stained tissue sections were examined under light microscope.

RESULTS

CD34 and c-kit were positive in the staining of capillary endothelial cells in tonsil and ductal cells in normal mammary tissues, respectively. Negative controls were all negative. Among the specimens of 32 cases of cholangiocarcinoma, CD34 was strongly positive in the staining of all capillary endothelial cells and negative in tumor cells (Figure 1). However, c-kit was positive in the staining of tumor cells in 1 case of cholangiocarcinoma originating from common bile duct (case 32, Table 1). This was an 80 years old patient with moderately differentiated cholangiocarcinoma, and about 5% of the tumor cells were positively stained at cell
membrane and cytoplasm (Figure 2). The positive result was repeatedly identified in several sections from the same specimen.

### Table 1 Clinical characteristic of the patients with cholangiocarcinoma

| No. | Sex | Age (yr) | Location of adenocarcinoma | Differentiation |
|-----|-----|---------|-----------------------------|---------------|
| 1   | F   | 78      | CBD                         | moderately    |
| 2   | F   | 68      | CBD                         | moderately    |
| 3   | M   | 63      | CBD                         | moderately    |
| 4   | M   | 67      | IHBC                        | moderately    |
| 5   | M   | 58      | IHBC                        | well          |
| 6   | M   | 49      | CBD                         | poorly        |
| 7   | M   | 80      | IHBC                        | well          |
| 8   | M   | 75      | CBD                         | poorly        |
| 9   | M   | 77      | IHBC                        | well          |
| 10  | F   | 74      | CBD                         | well          |
| 11  | M   | 50      | CHBD                        | well          |
| 12  | F   | 68      | CBD                         | well          |
| 13  | M   | 62      | IHBC                        | well          |
| 14  | F   | 69      | IHBC                        | well          |
| 15  | F   | 67      | IHBC                        | well          |
| 16  | M   | 52      | IHBC                        | moderately    |
| 17  | M   | 59      | IHBC                        | moderately    |
| 18  | F   | 74      | CBD                         | poorly        |
| 19  | M   | 64      | CHBD                        | well          |
| 20  | F   | 62      | IHBC                        | moderately    |
| 21  | F   | 68      | IHBC                        | poorly        |
| 22  | M   | 61      | CHBD                        | poorly        |
| 23  | F   | 46      | CBD                         | moderately    |
| 24  | F   | 73      | CBD                         | moderately    |
| 25  | F   | 62      | IHBC                        | well          |
| 26  | M   | 59      | CHBD                        | well          |
| 27  | M   | 64      | CBD                         | poorly        |
| 28  | M   | 76      | IHBC                        | moderately    |
| 29  | M   | 24      | IHBC                        | well          |
| 30  | M   | 56      | IHBC                        | moderately    |
| 31  | F   | 60      | CBD                         | moderately    |
| 32  | F   | 80      | CBD                         | moderately    |

F: Female; M: Male; CBD: Common bile duct; CHBD: Common hepatic bile duct; IHBC: Intrahepatic bile duct.

Liver stem-like cells are small and oval in shape with relatively large oval nuclei. They are immunoreactive for OV-6 (rat oval cell marker), cytokeratin (CK) 8 and CK 18 (both are epithelial cell markers), CK 7 and CK 19 (both are biliary cell markers), CK 14, and chromogranin-A[15,19]. Liver stem-like cells are heterogeneous, and could be classified into 3 types based on their differentiation characteristics. Type I represents the most undifferentiated cells, type II the progenitor cells differentiating towards biliary lineage, and type III the progenitor cells differentiating towards hepatic lineage[16]. In human liver, these liver stem-like cells have been found in focal nodular hyperplasia, hepatic adenoma, hepatocellular carcinoma and hepatoblastoma[13-16].

CD34 and c-kit are two markers of hematopoietic stem cells. However, recently in normal human liver, c-kit was detected in canal of Hering where the putative liver stem cells may exist[1]. In patients with fulminant hepatic failure, over expression of c-kit was detected in activated liver stem-like cells[20]. CD34 was also identified in rat liver stem-like cells[21]. Using immunomagnetic separation method, CD34 and c-kit positive cells were isolated from human liver. These cells were able to proliferate and differentiate into both biliary epithelial and endothelial cells in vitro[19]. This suggested that CD34 and c-kit positive cells in human liver might represent biliary progenitor cells, biliary and endothelial cells might share the same progenitor cells. Among the 12 cases of hepatoblastoma, Ruck et al. reported that CD34 was found to be immunoreactive with both tumor cells and endothelial cells in 1 case of small cell hepatoblastoma, and this indicated the possible stem cell origin of hepatoblastoma[22].

In this study, a few tumor cells in 1 of 32 cases of cholangiocarcinoma were immunoreactive with c-kit, and this suggested their possible origin of biliary stem cells.

As putative liver stem cells possibly exist in terminal bile ductules (canal of Hering), intrahepatic cholangiocarcinoma is supposed to originate from liver stem cells more likely than extrahepatic cholangiocarcinoma. However, the cells with stem cell characteristics may not only exist in intrahepatic bile ducts, but also in extrahepatic bile ducts. In embryonic development of rat liver, both intra- and extrahepatic bile ducts originated from AFP- and albumin-containing hepatoblasts[23]. The remnant of embry liver stem cells may also exist in extrahepatic bile ducts. In human, it was observed that hepatocellular carcinoma could also develop from extrahepatic bile ducts[24-26]. These tumors might originate from liver stem cells in extrahepatic bile ducts by maturation arrest. Thus, extrahepatic cholangiocarcinoma that was immunoreactive with c-kit in this experiment might also originate from putative liver stem cells.

Based on animal experiments, different carcinogenic regiments might act on different level of cells in hepatic lineage and produce hepatic carcinoma by different mechanisms[27]. Diethylnitrosamine acted on mature hepatocytes and induced hepatocellular carcinoma by de-differentiation. Furan injured bile duct progenitor cells and induced cholangiocarcinoma by maturation arrest. 2-aceylaminofluorene acted on ductular bipolar progenitor cells and induced hepatocellular carcinoma by maturation arrest. In choline deficiency models, the periductular stem cells could be activated to induce hepatocellular carcinoma by maturation arrest. The exact causes of human cholangiocarcinoma are still unclear, and there may be more than one mechanism of its carcinogenesis. In this study, only a few tumor cells in 1 case of cholangiocarcinoma were c-kit immunoreactive, and we could
not draw a sound conclusion. Tumor cells may lose their markers of stem cells during maturation arrest. Thus, further researches, such as increasing the number of cases and discovering new biliary progenitor cell markers, are needed to answer if human cholangiocarcinoma originates from stem cells.

ACKNOWLEDGEMENTS

The authors thank Professor Andrea Frilling at Department of General Surgery and Transplantation, University Hospital Essen, Germany, for the revision of the manuscript.

REFERENCES

1. Sell S. Cellular origin of cancer: dedifferentiation or stem cell maturation arrest? Environ Health Perspect 1993; 101(Suppl 5): 15-26
2. Sell S. Is there a liver stem cell? Cancer Res 1990; 50: 3811-3815
3. Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, Kumar A, Crawford J. The canals of hering and hepatic stem cells in humans. Hepatology 1999; 30: 1425-1433
4. Zhang Y, Bai XF, Huang CX. Hepatic stem cells: existence and origin. World J Gastroenterol 2003; 9: 201-204
5. Thorgeirsson SS. Hepatic stem cells. Am J Pathol 1993; 142: 1331-1333
6. Sigal SH, Brill S, Fiorino AS, Reid LM. The liver as a stem cell and lineage system. Am J Physiol 1992; 263(2 Pt 1): G139-G148
7. Alison M, Sarraf C. Hepatic stem cells. Hepatology 1998; 29: 676-682
8. Haque S, Haruna Y, Saito K, Nalesnik MA, Atillasoy E, Thung SN, Gerber MA. Identification of bipotential progenitor cells in human liver regeneration. Lab Invest 1996; 75: 699-705
9. Lowes KN, Brennan BA, Yeoh GC, Olynyk JK. Oval cell numbers in human chronic liver diseases are directly related to disease severity. Am J Pathol 1999; 154: 537-541
10. Crosby HA, Hubshcer S, Fabreis L, Joplin R, Sell S, Kelly D, Strain AJ. Immunolocalization of putative human liver progenitor cells in livers from patients with end-stage primary biliary cirrhosis and sclerosing cholangitis using the monoclonal antibody OV-1 and OV-6. Am J Pathol 1998; 152: 771-779
11. Hsia CC, Evarts RP, Nakatsukasa H, Marsden ER, Thorgeirsson SS. Occurrence of oval-type cells in hepatitis B virus-associated human hepatocarcinogenesis. Hepatology 1992; 16: 1327-1333
12. Ma X, Qiu DK, Peng YS. Immunohistochemical study of hepatic oval cells in human chronic viral hepatitis. World J Gastroenterol 2001; 7: 238-242
13. Roskams T, De Vos R, Desmet V. ‘Undifferentiated progenitor cells’ in focal nodular hyperplasia of the liver. Histopathology 1996; 28: 291-299
14. Libbrecht L, De Vos R, Cassiman D, Desmet V, Aerts R, Roskams T. Hepatic progenitor cells in hepatocellular adenomas. Am J Surg Pathol 2001; 25: 1388-1396
15. Wu PC, Lai VC, Fang JW, Gerber MA, Lai CL, Lau JY. Hepatocellular carcinoma expressing both hepatocellular and biliary markers also expresses cytokeratin 14, a marker of bipotential progenitor cells. J Hepatol 1999; 31: 965-966
16. Ruck P, Xiao JC, Pietsch T, Von Schweinitz D, Kaiserling E. Hepatic stem-like cells in hepatoblastoma: expression of cytokeratin 7, albumin and oval cell associated antigens detected by OV-1 and OV-6. Histopathology 1997; 31: 324-329
17. Lee JH, Rim HJ, Sell S. Heterogeneity of the “oval-cell” response in the hamster liver during cholangiocarcinogenesis following clonorchis sinensis infection and dimethyl-nitrosamine treatment. J Hepatol 1997; 26: 1313-1323
18. Crosby HA, Kelly DA, Strain AJ. Human hepatic stem-like cells isolated using c-kit or CD34 can differentiate into biliary epithelium. Gastroenterology 2001; 120: 534-544
19. Roskams T, De Vos R, Van Eyken P, Myazaki H, Van Damme B, Desmet V. Hepatic OV-6 expression in human liver disease and rat experiments: evidence for hepatic progenitor cells in man. J Hepatol 1998; 29: 455-463
20. Baumann U, Crosby HA, Ramani P, Kelly DA, Strain AJ. Expression of the stem cell factor receptor c-kit in normal and diseased pediatric liver: identification of a human hepatic progenitor cell? Hepatology 1999; 30: 112-117
21. Omori N, Omori M, Evarts RP, Teramoto T, Miller MJ, Hoang TN, Thorgeirsson SS. Partial cloning of rat CD34 cDNA and expression during stem cell-dependent liver regeneration in the adult rat. Hepatology 1997; 26: 720-727
22. Ruck P, Xiao JC, Kaiserling E. Immunoreactivity of sinusoids in hepatoblastoma: an immunohistochemical study using lectin UEA-1 and antibodies against endothelium-associated antigens, including CD34. Histopathology 1995; 26: 451-455
23. Shiojiri N, Lemire JM, Fausto N. Cell lineages and oval cell progenitors in rat liver development. Cancer Res 1991; 51: 2611-2620
24. Thomsen CH, Kruse A, Petersen A. Hepatocellular carcinoma presenting as a tumour of the hilar and extrahepatic bile ducts. Eur J Gastroenterol Hepatol 1998; 10: 803-804
25. Cho HG, Chung JP, Lee KS, Chon CY, Kang JK, Park IS, Kim KW, Chi HS, Kim H. Extrahepatic bile duct hepatocellular carcinoma without primary hepatic parenchymal lesions-a case report. Korean J Intern Med 1996; 11: 169-174
26. Park CM, Cha IH, Chung KB, Sul WH, Lee CH, Choi SY, Chae YS. Hepatocellular carcinoma in extrahepatic bile ducts. Acta Radiol 1991; 32: 34-36
27. Sell S. Cellular origin of hepatocellular carcinomas. Semin Cell Dev Biol 2002; 13: 419-424

Edited by Wang XL and Chen WW Proofread by Xu FM