

Immunohistochemical study of hepatic oval cells in human chronic viral hepatitis

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Abstract

AIM To detect immunohistochemically the presence of oval cells in chronic viral hepatitis with antibody against c-kit.

METHODS We detected oval cells in paraffin embedded liver sections of 3 normal controls and 26 liver samples from patients with chronic viral hepatitis, using immunohistochemistry with antibodies against c-kit, π class glutathione S-transferase (π -GST) and cytokeratins 19 (CK19).

RESULTS Oval cells were not observed in normal livers. In chronic viral hepatitis, hepatic oval cells were located predominantly in the periportal region and fibrosis septa, characterized by an ovoid nucleus, small size, and scant cytoplasm. Antibody against stem cell factor receptor, c-kit, had higher sensitivity and specificity than π -GST and CK19. About 50%-70% of c-kit positive oval cells were stained positively for either π -GST or CK19.

CONCLUSION Oval cells are frequently detected in human livers with chronic viral hepatitis, suggesting that oval cell proliferation is associated with the liver regeneration in this condition.

INTRODUCTION

Although essentially a quiescent organ, the normal adult liver can fully regenerate following surgical resection or injury. Liver regeneration is usually achieved by the entry of normally proliferatively quiescent, differentiated hepatocytes into the cell cycle, but, when hepatocyte regeneration is defective, oval cells can migrate outward from the portal tracts and then differentiate into hepatocytes^[1-3]. The term oval cells described as small cells with oval nuclei that arise in the periphery of the portal tracts in rat models of hepatocarcinogenesis and injury^[4-7]. These cells are thought to have both clonogenic and bipotential capacity, i.e., the ability to proliferate and differentiate into cells of either hepatocyte or biliary epithelial cells^[8]. There is also evidence that under certain conditions, oval cells can be induced to differentiate into non-hepatic lineages including intestinal and pancreatic epithelium^[9]. The origin of oval cells and their precise location within the liver have remained enigmatic^[10]. The aim of this study is to detect immunohistochemically the presence of oval cells in chronic viral hepatitis with antibody against c-kit.

MATERIALS AND METHODS

Tissue samples

Formalin-fixed, paraffin-embedded liver biopsy specimens from 26 patients with chronic liver diseases were obtained from the Department of Histopathology at Shanghai Institute of Digestive Diseases (Renji Hospital). Patient age ranged from 23 to 71 years, with mean age of 54 years. Twenty-six patients had been diagnosed having chronic viral hepatitis (twenty-one chronic hepatitis B; five chronic hepatitis C) with various degree fibrosis. There were mild fibrosis ($n = 5$), moderate fibrosis ($n = 8$), severe fibrosis ($n = 6$), and hepatic cirrhosis ($n = 7$). Three specimens of grossly normal liver tissues from the area surrounding benign angiomas were used as references. Specimens were fixed immediately in 10% neutral formalin and embedded in paraffin.

Primary antibodies

To highlight the presence of oval cells, three primary antibodies were used. The antibody against stem cell factor receptor, c-kit, was purchased from Oncogene Research Products. c-kit (Ab-1) is a purified rabbit polyclonal antibody raised against the

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peptide (GSTASSSQPLLVDV), a sequence found at the Carboxyterminus corresponding to residues 961-976. Antibodies against π -class glutathione S-transferase (π -GST, clone 353-10) and cytokeratins 19 (CK19, clone BA17.1) were purchased from Dako Co, Denmark.

Immunohistochemistry

Immunohistochemical staining was performed on serial sections at room temperature, using the alkaline phosphatase method. The sections were deparaffinized in xylene and rehydrated through graded alcohol. The sections were boiled in 6M urea at 95°C for 10 min for c-kit staining. Endogenous peroxidases were inactivated by immersing the sections in hydrogen peroxide for 10 minutes, then were incubated for 10 minutes with normal swine serum in Tris-buffered saline to block non-specific binding. The sections were subsequently incubated overnight at 4°C with relevant antibodies (1:100 dilution respectively). The following day, the sections were incubated with biotinylated anti-mouse or anti-rabbit IgG (1:50 dilution, Maxim Biotech Inc., USA) for 45 minutes, followed by peroxidase-conjugated streptavidin (1:50 dilution, Maxim Biotech Inc.). The chromogenic reaction was

developed with diaminobenzidine for 10 minutes, and all sections were counterstained with hematoxylin. Controls consisted of omission of the primary antibody.

RESULTS

Oval cells were not detected in normal liver tissue, but were detected in most liver tissues from patients with chronic viral hepatitis. Oval cells were characterized by ovoid nuclei from 7 $\mu\text{m} \times 9 \mu\text{m}$ to 12 $\mu\text{m} \times 17 \mu\text{m}$, small size, and scant cytoplasm (Figure 1). They were located predominantly in the periportal region (Figure 2) in hepatic cirrhosis, and were often found in close association with inflammatory cells in chronic active hepatitis (Figure 3). There were "transitional cells" in the parenchyma with size and structure between those of human oval cells and mature hepatocytes. They were moderately stained by CK19 antibody, had round nuclei, more cytoplasm, and were smaller in size than mature hepatocytes (Figure 4). c-kit antibody had higher sensitivity and specificity than π -GST and CK19. About 50%-70% of C-kit positive oval cells were stained positively for either π -GST or CK19. Some mature hepatocytes also expressed π -GST. Most mature bile ducts also expressed CK19.

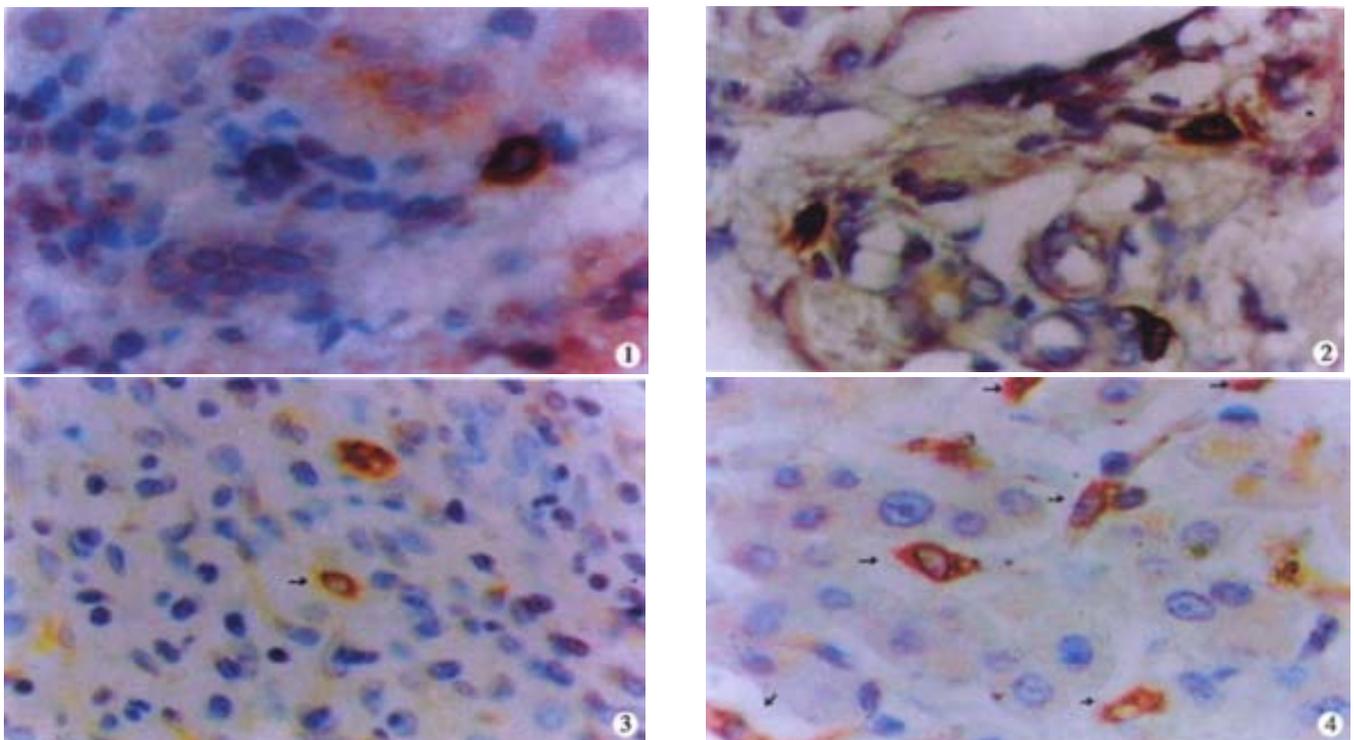


Figure 1 Oval cell identified by c-kit staining (Immunohistochemistry ABC method; original magnification: $\times 400$)

Figure 2 Oval cells were located predominantly in the periportal region in hepatic cirrhosis (Immunohistochemistry ABC method; stained by c-kit; original magnification: $\times 400$)

Figure 3 Oval cells were often found in close association with inflammatory cells in chronic active hepatitis (Figure 3). (Immunohistochemistry ABC method; stained by π -GST, original magnification: $\times 400$)

Figure 4 "Transitional cells" from a patient with hepatic cirrhosis (Immunohistochemistry ABC method; stained by CK19; original magnification: $\times 400$)

DISCUSSION

Hepatic oval cells proliferate under certain conditions, mainly when hepatocytes are prevented from proliferating in response to liver damage, and may be stem cells of hepatocytes and bile duct cells or the intermediate progeny of a hepatic stem cell^[2]. Oval cells in animals are activated following administration of a variety of toxins and carcinogens alone or combined with other surgical or dietary regimens^[11-19]. One of the models studied most is acetylaminofluorene treatment followed by partial hepatectomy, and an array of cytokines and growth factors have been shown to be an up-regulatory mechanism, is being delineated, for example interferon γ is implicated in orchestrating the process^[20]. The oval cell itself however, probably represents the activated progeny of a dormant stem cell compartment although oval cells are readily identified in injury liver, one area of great controversy is the question of where these putative stem cells reside in the normal liver^[3]. One suggestion is that they are present in the canals of Hering, that is the region where cells are transitional between the periportal hepatocytes and the biliary cells lining the smallest terminal bile duct^[21]. Others suggest that there are cells which are located in the portal tracts, in the periductular region, or even that periportal hepatocytes have stem cell or metaplastic properties^[2].

Oval cells are of great clinical interest since they may be the progenitor cells of both hepatocellular carcinomas and cholangio-carcinomas. Furthermore, they could be useful vehicles for *ex vivo* gene therapy for the correction of metabolic liver diseases^[2]. However, identifying stem cells or their progeny in human liver has been a challenge. Oval cells, similar in morphology and antigenic profile to those seen in rodents, may be associated with many liver diseases in humans. These include submassive necrosis, focal nodular hyperplasia, primary biliary cirrhosis and primary sclerosing cholangitis, alcoholic hepatitis and cirrhosis, hepatoblastoma and HBV-associated hepatocellular carcinoma, genetic hemochromatosis, and hepatitis C^[22-30]. We detected the presence of hepatic oval cell in patients with chronic viral hepatitis. The human oval cell has a distinct morphology, with oval-shaped nuclei, small size and scant cytoplasm, and thus can be distinguished from the mature hepatocytes and inflammatory cells^[1]. Oval cells express a wide variety of antigens that can be detected immunocytochemically. Although by no means specific for oval cells, many antibodies are very highly expressed, and can be used to highlight the presence of oval cells in histological sections^[2].

Stem cell factor (SCF), a cytokine with

structural similarity to the colony stimulating factors, was first isolated from conditioned medium of Buffalo rat liver cells^[31]. Since then, it has become clear that SCF is critically important for early epithelial stem cell differentiation in hematopoiesis^[32] or gametogenesis^[33]. The corresponding receptor for SCF is encoded by the proto-oncogene *c-kit*, and exhibits a tyrosine kinase activity facilitated by its intracellular domain^[34]. Target cells for SCF, which express *c-kit*, include mast cells^[35], hematopoietic progenitor cells^[32], and germ cells^[33]. Recent studies suggest that SCF and *c-kit* may be involved in early growth and development of hepatic progenitor cells. Up-regulation of SCF and *c-kit* has been described in AAF-treated, partially hepatectomized rats, in which the carcinogen treatment inhibits the replication of hepatocytes and the induction of oval cells may be observed^[19,36]. A similar increase of SCF and *c-kit*, accompanied by oval cell proliferation, has also been reported in bile duct-ligated rat^[37]. Baumann *U et al* suggests that *c-kit*-positive cells may represent a hepatic progenitor cell population in normal and diseased pediatric liver^[38]. In our study, we observed that some *c-kit*-positive cells with an oval-like morphology were present in human livers with chronic viral hepatitis, and that antibody against *c-kit* is useful in detecting the oval cells. We detected π -GST as a fetal GST in the majority of oval cells, supporting the view that oval cells display characteristics which resemble fetal hepatocytes or liver stem cells^[29,39]. CK19n positive staining suggests that oval cells have characteristics of biliary epithelium^[40]. The combined use of these antibodies and the knowledge of morphology allows us to reliably identify oval cells.

As hepatitis develops, hepatocyte necrosis is followed by an attempted secondary proliferation response of mature hepatocytes, but this proliferation response is often impaired in chronic liver diseases. Thus, oval cells may have a chance to proliferate and differentiate into hepatocytes or biliary epithelium cells^[41]. We observed that oval cells were located predominantly in the periportal region, and were found in close association with fibrosis septa and inflammatory infiltrates. This location suggests that cytokines or other factors associated with the development of inflammation and fibrosis may be required to stimulate oval cell proliferation, differentiation and migration^[42]. The matrix in fibrotic and cirrhotic livers is the binding site for both epidermal growth factor^[43,44] and hepatocyte growth factor^[45], whose receptors have been shown to be involved in proliferation of oval cells^[46]. Additionally, the presence of "transitional

cells” may be evidence that oval cells are progenitors of hepatocytes.

While the debate on the source and location of hepatic stem cells is ongoing, two recent papers add a new dimension and offer a challenging alternative hypothesis to explain the origin of oval cells. By transplanting rat bone marrow into lethally irradiated recipients and following the fate of syngeneic cells using various markers, Petersen BE et al^[47] reported striking changes in the livers of animals induced to regenerate following 2-AAF and CCl₄ treatment. Male donor marrow cells were visualized in female recipients. In a second model, marrow from dipeptidyl peptidase IV positive animals was transplanted into dipeptidyl peptidase IV deficient animals. In both cases, evidence was presented to suggest that the donor cells migrated into the livers of recipient animals and subsequently underwent differentiation to become hepatocytes, although it was less clear whether ductular cells of biliary phenotype developed^[47]. A second recently published study describes a similar approach comprising a mouse marrow transplant model which, interestingly, did not include a liver injury step^[30]. This new report provides confirmatory evidence that bone marrow derived haematopoietic stem cells can indeed give rise to hepatocytes^[48]. The ability to identify and exploit a human hepatic clonal stem cell could have important clinical implications, since generating large numbers of differentiated and therefore fully functional human hepatocytes has enormous potential^[49,50].

In conclusion, the presence of oval cells in human livers with chronic viral hepatitis indicates that oval cell proliferation may be one of the mechanisms in liver regeneration in this condition. The origin, growth, and differentiation of this cell is worth investigating further.

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