Original article

The role of oval cells in chronic hepatitis type B and type C. A clinicopathologic study

A.C. Tsamandas[1], E.S. Felekouras[2], Stavroula Salakou[3], Th. Kourelis[1], K. Tepetes[2], D.S. Bonikos[1], E. Papalampros[2], G.K. Michalopoulos[3]

SUMMARY

Aim: **Oval hepatocytes, (OC) as a part of liver stem cells, are involved in the progress of liver disease and hepatocellular carcinoma (HCC) development, in experimental models. This study investigates the presence and possible role of OC in chronic hepatitis (CH) type B and C in humans.**

Design: **The study comprised 50 needle liver biopsies with CH and 50 wedge/needle normal liver biopsies as controls. CH cases included 29 HBV (all HBSAg+-14HbcAg+), and 21 HCV (PCR+) instances. HAI ranged from 3/18-15/18, and the architectural stage from 1 to 6 (6HBV/8HCV). Epidemiologic and laboratory data were obtained from medical records. The streptavidin-biotine technique was employed on paraffin sections using antibodies for cytokeratin 19 (CK19), glutathione-S-transferase-π (GST-π) and leukocyte common antigen (LCA). Cells with microscopic features of OC that were CK19 (+), or GST-π (+) and LCA (-) were scored. Oval cell percentage was expressed following morphometric analysis, and the results were correlated with clinical parameters, grade and stage of CH.**

Results: **Oval cells were present in all 50 liver biopsies with CH but in 0/50 controls. They were located in periportal areas; occasionally formation of small ducts was recorded. Oval cells were also recognized more often in association with fibrous bands and accompanied inflammatory infiltrates. The CK19+ cells varied from 20-90% and GST-π+ cells from 20-75%, of the total oval cells numbers. The percentage of CK19+ and GST-π+ cells were directly correlated with i) categories A, B, and D of Ishak score ($p<0.01$), ii) total Ishak score ($p<0.01$), iii) architectural grade ($p<0.01$), iv) transaminases values ($p<0.05$). Direct correlation was also seen between CK19 (+) and GST-π(+) cells ($p<0.05$).**

Conclusions: Presence of oval hepatocytes is a common finding in liver biopsies from patients with CH type B or C. The significant association between oval cell percentage and severity of disease (HAI grade and stage) implies that OC proliferation is related to progress of liver disease and/or increased risk for hepatocellular carcinoma development.

Key words: oval cell, chronic hepatitis, carcinogenesis, fibrosis

Department of Pathology[1] University of Patras School of Medicine, Patras Greece, First Surgical Clinic[2] University of Athens, Athens Greece, Department of Pathology[3] University of Pittsburgh School of Medicine, Pittsburgh PA, USA

Author for correspondence:

Athanassios C. Tsamandas, MD, Department of Pathology University of Patras School of Medicine, 88 Agiou Demetriou str., 262 25 Patras, Greece, Tel: +30-61-271238, Fax: +30-61-994202, e-mail: tsamanda@otenet.gr

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common human malignancies and its risk of development is significantly increased in chronic liver disease, especially in instances of chronic hepatitis type C and when cirrhosis is present.[1-5] After cirrhosis is established, liver regeneration following hepatocyte necrosis, is mediated by mature liver cell proliferation and alternatively by stem cell proliferation and differentiation into hepatocytes.[6-9]

The origin of hepatic stem cell that produces progeny called oval cell in the adult liver of humans and animals, has been the subject of extensive research over the past several decades. Activation of the hepatic stem cell

compartment is histologically detectable only when hepatocyte proliferation is suppressed.[10,11] Oval cells are seen to arise in the periportal region of the liver and morphologically are small in size (approximately 10μm) and have a large nucleus-to-cytoplasmic ratio, with an oval shaped nucleus.[12] They have similarities to bile duct cells and hepatocytes with distinct isoenzyme profiles, expressing high levels of α-fetoprotein (AFP), certain keratin markers (cytokeratin 19-CK19), and γ-glutamyl transpeptidase.[18,13-16] Immunohistochemical markers include antibodies such as OV6, CK19, GST-π, MK-2, and AFP.[17-21] High levels of certain mRNAs like AFP and stem cell factor can also be expressed by oval cells.[20]

Several previous studies have documented the emergence of oval cells following chemical injury, such as with ethionine and with galactosamine.[7,22-24] Studies in rodent models of hepatocarcinogenesis suggest that activation of oval cells could be the mechanism by which the liver may replace destroyed parenchyma in chronic liver disease.[6-9,25,26] In addition, experiments in animals exposed to a variety of carcinogenic regimens showed a uniform pattern of preneoplastic changes ultimately giving rise to HCC. In humans, oval cells have been reported in hepatitis B-associated HCC and chronic liver disease that associated with ductular proliferation.[19,27,28] We performed the study mentioned in this report to detect the presen-ce of oval cells in cases of chronic hepatitis B or C severity and determine a possible relationship between the severity of liver disease and the number of oval cells.

Table 1. Clinical and histologic characteristics of patients (N=100)

	HBV	HCV
		29 21
Sex	**19M; 10F**	**16M; 5F**
Age (yr)		
Mean (range)	52 (30-70)	49 (25-60)
HAI score, mean (range)		
Category A	3 (1-4)	2 (1-4)
Category B	1 (0-3)	1 (0-3)
Category C	2 (1-4)	2 (1-4)
Category D	3 (1-4)	3 (1-4)
Total score	9 (3-15)	8 (3-15)
Stage		
1	8	3
2	4	2
3	4	2
4	4	3
5	3	3
6	6	8
Laboratory values mean (range)		
AST	163 (98-205)	144 (91-198)
ALT	177 (90-210)	162 (89-201)

CH: chronic hepatitis, HBV: hepatitis B, HCV: hepatitis C, HAI: hepatitis activity index

MATERIALS AND METHODS

Patients

The study comprised 50 patients with chronic hepatitis (CH) proven by liver biopsy and diagnosed according to standard criteria.[29] They were 29 HBV (all HBSAg+-14HbcAg+), and 21 HCV (PCR+) instances. For the purposes of the study, all the epidemiological, clinical, and biochemical features pertaining these patients at the time of the diagnosis, were obtained by review of the clinical records, and are listed in table 1.

Methods

Conventional pathologic design

All liver tissues obtained by needle biopsy (14G) were fixed in 10% neutral formalin, embedded in paraffin and examined in multiple levels (10 sections of 4 μm each). Histological study included hematoxylin and eosin (H&E), periodic acid Schiff (PAS) for bile duct evaluation), Masson's trichrome and reticulin. Several histologic features were assessed in each biopsy in a blind fashion, and finally the Hepatitis Activity Index (HAI) was applied and the architectural grade was recorded in each instance.[30] For control purposes 50 wedge or needle biopsies from normal liver parenchyma were used. These were obtained from 50 patients during operation for cholelithiasis, without co-existing choledocholithiasis.

Immunohistochemistry

All 100 liver biopsies (50 with CH and 50 controls) included in the study were stained for presence or absence of GST-π, CK19 and LCA antigens, using a standard streptavidin-biotin immunohistochemical method. For CK19 expression microwave antigen retrieval was used. Primary antibodies used included: monoclonal mouse antibody to CK19 (Novocastra UK), in a dilution 1:80, polyclonal antibody GST-π (Novocastra, UK), in a dilution of 1:80 and monoclonal antibody to LCA (Biogenex, UK) ready for use.

Measurement of oval cell proliferation

Each liver biopsy was scanned in a low power and

cells that fullfilled the morphologic criteria of oval cells and displayed cytoplasmic positivity to CK19 or GST-π but were negative for LCA, were scored. Cell counts were performed manually at a X400 magnification using a 10x10-microscope grid. Both the numbers of immunoreactive cells and the total number of oval cells (at least 500 cells) were determined by visual inspection of five non-overlapping different fields per section. The percent of immunoreactive (CK19+ or GST-π+ cells) was based on the ratio of positive cells to the total number of cells counted. The variance in oval cell counts from section to section in the same biopsy was <10%. The average of these scores was then taken.

Statistical analysis

Results were expressed as the mean ± and standard deviation. The Mann Whitney test was employed to determine whether the differences between groups were significant. A p-value < 0.05 was set as significant.

RESULTS

Liver pathology

All 50 needle liver biopsies included in the study, contained more than 4 portal tracts and considered sufficient for pathologic evaluation. Table 1 demonstrates the value of each HAI parameter, total HAI score and HAI stage for chronic hepatitis instances. Total HAI score ranged from 3/18 (3HBV and 2HCV cases) to 15/18 (4HBV and 2HCV cases). Evaluation of Masson's trichrome and reticulin stain revealed HAI stage 6 in 6HBV and 8HCV cases.

Immunohistochemical results

Oval cells (CK19+ or GST-π+ and simultaneously LCA negative cells) were present in all 50 biopsies with chronic hepatitis but in none of the control biopsies. They were located in periportal areas and occasionally formed ductular structures (Fig 1, 2). Oval cells were found in close proximity with fibrous septa, surrounding regenerative nodules, or associated with inflammatory cell infiltrates (Fig 3). GST-π was also expressed in mature hepatocytes (Fig 4). The number of CK19 positive oval varied from 20±2 to 90±8 in cases of chronic hepatitis type B and from 22±4 to 89±6 in chronic hepatitis type C instances. The number of GST-π positive oval cells varied 20±2 to 70±5 in cases of chronic hepatitis type B and from 21±4 to 75±3 in chronic hepatitis type C instances.

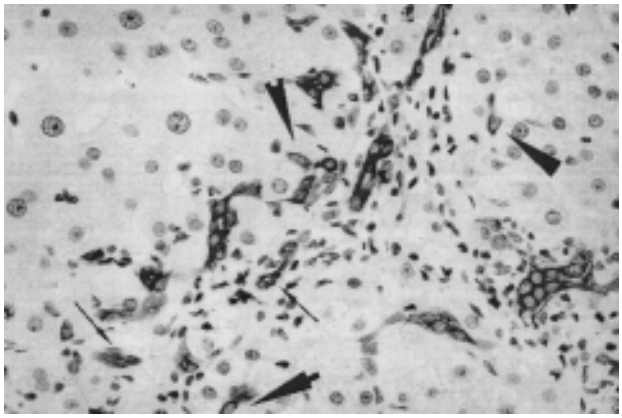

Figure 1. Photomicrograph of immunohistochemistry for CK19+ oval cells (thick arrows) that form ductular structures (thin arrows) in a case of chronic hepatitis type B (ABC x400).

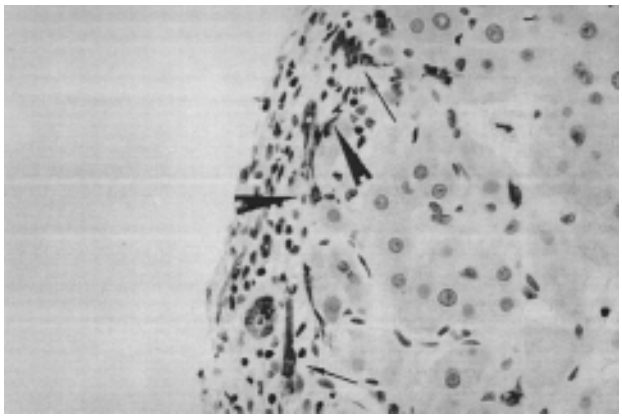

Figure 2. Photomicrograph of immunohistochemistry for GST-π+ oval cells (thick arrows) that form ductular structures (thin arrows) in a case of chronic hepatitis type B (ABC x 400).

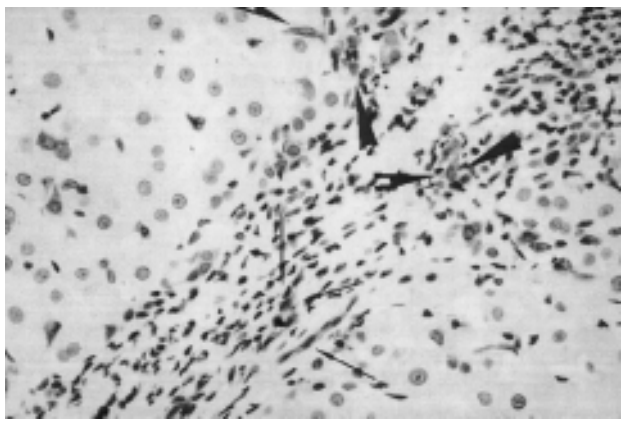

Figure 3. Photomicrograph of immunohistochemistry for CK19+ oval cells (thick arrows) present within fibrous septa, that accompany lymphocytic inflammation in a case of chronic hepatitis type C. Note that oval cells form ductular structures (thin arrows). (ABC x 200).

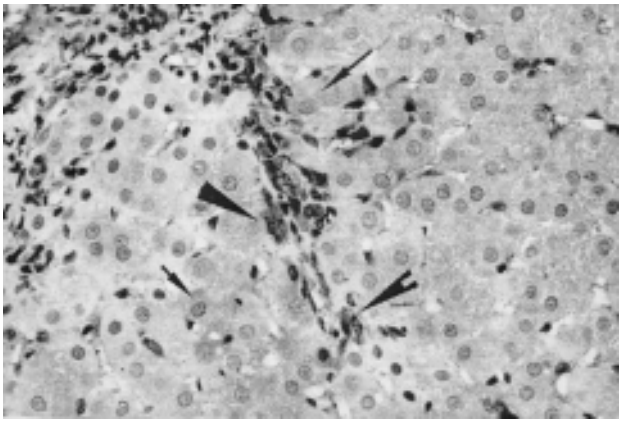

Figure 4. Photomicrograph of immunohistochemistry for GST-π- expressed within oval cells (thick arrows) and hepatocytes (thin arrows) (ABC x 200).

Correlation between parameters

Positive correlations were observed between CK19+ oval cells and i) categories A, B, and D of HAI score ($p<0.01$ in each instance), ii) total HAI score ($p<0.01$), iii) architectural grade ($p<0.01$), iv) transaminases values ($p<0.05$ in each instance). Similar correlations observed between GST-π+ cells with each parameter of HAI score, total HAI score, hepatitis grade and transminases values. In addition the numbers of CK19+ cells were positively correlated with GST-π+ cells ($p<0.05$). No significant correlation ($p>0.05$) was recorded between CK19+ and GST-π+ cells and cause of hepatitis when the values were matched for each parameter of HAI, total HAI or hepatitis grade.

DISCUSSION

This study demonstrates that oval cells are frequently present in patients with chronic hepatitis B or C. In addition, oval cell numbers increase significantly with progression of disease severity (grade and stage) in both groups studied, suggesting that oval cell proliferation is not disease-specific but occurs in response to progressive liver injury and fibrosis. This concurs with the hypothesis that oval cell activation is associated with increased risk for development of HCC with advancing liver disease, particularly when cirrhosis is present.[19]

The nature, site, and cell type origin of the "oval cells" that emerge in the liver after different types of injury has been a subject of several studies.[8,10,12-18,20] The appearance of both bi-liary and hepatocytic markers in these cells has reinforced the concept of the hepatic stem cell, and many investigators in the field consider these oval cells as the immediate progeny of a putative hepatic stem cell.18 Chemical injury to the liver induced by a variety of agents such as galactosamine and ethionine cause an increase in the numbers of oval cells.[7,22,24]

In animal models oval cell proliferation is associated with the presence of inflammatory cells within the liver.[26,31] When oval cells are isolated from animals placed on a choline-deficient, ethionine-supplemented diet, they rapidly deteriorate in culture suggesting that exogenous factors are required for their survival and proliferation.[26] It has been referred that oval cells presence is reduced in cases of experimental selective periportal damage even when mature hepatocyte proliferation has been inhibited.[11,24] In this and other previous studies, oval cells were often found in close association with fibrous tissue in the liver, often proliferating along the tracts from the expanded portal regions, and along the limiting plates surrounding regenerative nodules.[19] In addition, oval cells were often observed in close association with inflammatory cells, in patients with chronic hepatitis B or C. These observations suggest that cytokines or other factors produced by inflammatory cells or cells associated with the development of fibrosis, such as liver myofibroblasts and Kupffer cells, may be required to stimulate oval cell proliferation and migration.

Oval cell detection in human liver diseases is based on the presence of cells with the typical histopathological appearance combined with appropriate immunohistochemical markers. Several immunohistochemical markers have been described for oval cells including OV6, CK19, GST-π, MK-2, and AFP.[17-21,32] The combined use of immunohistochemistry and morphology to characterize oval cells, and the exclusion of inflammatory cells (LCA negative cells) in serial sections) lead to reliable results regarding oval cells presence. This study shows that GST-π and CK19 are reliable markers for oval cells. The numbers of CK19+ oval cells were higher when compared with GST-π+ cells (when the cases were matched for other histopathologic features), and this may reflect the heterogeneity of the oval cell population in terms of their developmental maturity or their commitment to either the hepatocytic or biliary lineage.

The significance of the ductule-like structures formed by oval cells in various disease states has not yet been clarified. Previous studies in rodents have demonstrated that oval cells can proliferate and form ductular-like structures during carcinogenesis and biliary obstruction.[25,26,32] Some of the cells in the ductule-like structures also express the adult L-pyrouvate kinase isoenzyme (L-PK), with a small population coexpressing M2-PK and

L-PK.[25,26,32] These observations suggest that some cells within the ductular structures may be capable of progressing along the hepatocyte lineage. Formation of ductal structures has been observed during liver regeneration following selective periportal necrosis after pretreatment with inhibitors of mature hepatocyte proliferation.[11,24] Possible explanations could be that a) the forerunners of the oval cells cannot "colonize" the immediate periportal site and lead to the oval cell response due to the damage of the overall tissue environment and b) that the canals of Herring (considered by most investigators as the compartment of biliary epithelium most likely to be associated with the oval cell response) are also damaged as "innocent bystanders" in chronic hepatitis cases as the disease progresses.

In conclusion, this study demonstrates that presence of oval cells is a common finding in liver biopsies from patients with chronic hepatitis type B or type C. The significant association between oval cell percentage and severity of disease (HAI grade and stage) implies that proliferation of those cells is related to progress of liver disease and/or increased risk for hepatocellular carcinoma development.

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