DYNAMIC CHANGES OF ITO CELLS IN EXPERIMENTAL LIVER CIRRHOSIS OF RAT

Zhao Jingmin* 赵景民 Zhang Yuee 张月娥 Wang Sinhe 王新禾
Zhai Weirong 翟为荣 Zhu Tengfang 朱腾方 Ling Yuqin 凌玉琴
Ying Yueying 应越英

Department of Pathology, Shanghai Medical University, Shanghai 200032

That Ito cells in rat liver express desmin was confirmed by immunohistochemical technique. Consequently, changes of desmin-positive cells, lysozyme-positive cells and fibronectin were further studied in experimental cirrhosis of rat. It was found that desmin-positive cells, with the transitional feature between Ito cells and myofibroblasts or fibroblasts under electron microscope, increased in number and expression of desmin in the necrotic areas as well as in the cellular fibrous septa, but decreased in number in the fibrous septa except those areas closed to the edges of the septa. These results suggested that Ito cells, myofibroblasts and fibroblasts might belong to the same cellular system and play an important role in the pathogenesis of cirrhosis. Meanwhile, it was also noted that changes of both fibronectin and lysozyme-positive cells were correlated with those of desmin-positive cells. These provide evidence that fibronectin and Kupffer cells might exert certain effects on the migration and proliferation of Ito cells in liver cirrhosis.

Key words: Ito cell, Cell differentiation, Liver cirrhosis, Experimental, Kupffer cell, Morphometry, Proliferation.

MATERIALS AND METHODS

Animal and Experimental Model

One hundred and thirty-five adult male Wistar

* Present Address:
Department of Pathology, Binzhou Medical College,
Binzhou 256603, Shandong Province, China
rats (Animal Center of Shanghai Medical University) were divided into nine experimental groups and nine control groups by chance. The rats in the experimental groups, ten rats in each group, were on standard diet and ground corn diet only for each week alternatively to induce low acetylcholine intake. Meanwhile the rats received 0.3 ml of 70% CCl₄ in olive oil of first dose and 50% CCl₄ thereafter per 100 gram of body weight subcutaneously for twice a week. The rats in control groups, five rats in each group, were given the same treatment as did in the experimental rats except injecting olive oil only. The animals were sacrificed on weeks 2, 4, 6, 8, 12, 14, 18 and 22 for one group each. The liver tissue were treated as follows: (a) fixed in 10% buffered formaldehyde and processed routinely for paraffin sections in 4 μm thickness for hematoxylin and eosin, reticulin (Gordon Sweet, Gs stain) and collagen (Van Gieson stain) and for immunohistochemical stains with peroxidase anti-peroxidase (PAP) method for labeling fibronectin and lysozyme. (b) Quickly frozen by isopropanol. Frozen sections were cut in 6 μm thickness and stained for desmin by PAP method. The antibody of rabbit anti-chick gizzard desmin and rabbit anti-human lysozyme were purchased from Dako corp., rabbit anti-rat fibronectin, goat anti-rabbit IgG and rabbit PAP were produced by our department. (C) fixed in buffered 1% OsO₄ and dehydrated through graded series of ethanol and acetone and embedded in Epon 812. The ultrathin sections were stained with lead citric acid and uranyl acetate and examined under Hitachi 500 transmission electron microscope.

Quantification of desmin-positive cells, positive areas of fibronectin and collagen fibers were performed by using image analysis system (Q520 Cambridge Corp.). Five microscopic fields including the four corners and one central part of each section were chose for morphometry. Each microscopic field was focused at either a necrotic region or a cellular fibrous septum or a fibrous septum. Which was carefully adjusted to the centre of the field with an appropriately grey level. At least five cases in each group were quantified automatically. The cell count of Ito cells and the positive areas of fibronectin and collagen fibers were treated by using quadratic variance analysis statistically.

RESULTS

Light Microscopic Study

The livers of control rats had no any changed. All rats in the experimental groups showed various degrees of fatty changes, hydrophic degeneration and focal necrosis of hepatic cells within 2 to 4 weeks. The "blood vessel septa" were formed and extended from lobules to lobules, which mainly consisted of the congestive hepatic sinusoids by the periphery of the necrotic liver cells. Following the increase of non-hepatic cells and extracellular matrix the "blood vessel septa" turned to be the cellular fibrous septa which gradually separated the hepatic lobules completely or in completely (Figure 1). Typical cirrhosis of the liver was occurred in 18 to 22 weeks with the fibrous septa which showed plenty of collagen fibers with VG staining surrounded regenerative nodules.

Immunohistochemical and Quantitative Studies

Desmin-positive cells in the livers of the control animals appeared stellate, spindle and linear in figure, which might contain lipid vesicles and localized in the perisinusoidal spaces evenly. The mean number of Ito cells in liver parenchyma was