Pathomorphological study on location and distribution of Kupffer cells in hepatocellular carcinoma

Kai Liu, Xu He, Xue-Zhong Lei, Lian-San Zhao, Hong Tang, Li Liu, Bing-Jun Lei

AIM: To clarify the location and distribution of Kupffer cells in hepatocellular carcinoma (HCC), and to investigate their role in hepatocarcinogenesis.

METHODS: Kupffer cells were immunohistochemically stained by streptavidin-peroxidase conjugated method (S-P). The numbers of Kupffer cells in cancerous, para-cancerous and adjacent normal liver tissues of 48 HCCs were comparatively examined.

RESULTS: The mean number of Kupffer cells in cancerous, para-cancerous and adjacent normal liver tissues was 12.7±6.8, 18.1±8.2 and 18.9±7.9 respectively. The number of Kupffer cells in cancerous tissues was significantly lower than that in para-cancerous tissues (t=2.423, P<0.05) and adjacent normal liver tissues (t=2.521, P<0.05). As tumor size increased, the number of Kupffer cells in cancerous tissues significantly decreased (F=4.61, P<0.05). Moreover, there was also a significant difference in the number of Kupffer cells among well-differentiated, moderately-differentiated and poorly-differentiated cases (F=4.49, P<0.05).

CONCLUSION: This study suggests that decrease of Kupffer cells in HCCs may play an important role in the carcinogenesis of HCC, the number of Kupffer cells in HCC is closely related to the size and differentiation grade of the tumor.

INTRODUCTION

Kupffer cells are important in maintaining homeostasis and in host anti-tumor defense mechanism[1-5]. It is thought that Kupffer cells are resident macrophages in the liver and are one kind of the sinusoidal endothelial cells[6]. Previously, Kupffer cells were not considered to exist in hepatocellular carcinoma (HCC) tissues. Recently, some studies revealed that Kupffer cells were also present in early-stage and well-differentiated HCC. Because small and well-differentiated HCCs could maintain an environment similar to that of normal sinusoids, Kupffer cells may also exist in these HCCs[7,8].

Unfortunately, whether Kupffer cells could exist in poorly-differentiated or large HCCs remains unidentified. Nevertheless, the difference of Kupffer cells among variable tissue types is not clear. The relationship between the decrease of Kupffer cells and carcinogenesis of HCC also needs to be clarified.

In the present study, we pathomorphologically examined the localization and distribution of Kupffer cells in 48 cases of HCCs samples embracing cancerous tissues, corresponding para-cancerous tissues and adjacent normal liver tissues.

MATERIALS AND METHODS

Tissues and specimen

Tissues of forty-eight primary HCCs including cancerous tissues, corresponding para-cancerous tissues and adjacent normal liver tissues were obtained with the informed consent of patients who underwent hepatectomy at the West China Hospital of Sichuan University. The surgically resected tissues were fixed in 10% formalin, embedded in paraffin, cut into 5 µm sections and stained with hematoxylin-eosin. Histopathological diagnosis and classification were made by the same pathologist.

Immunohistochemical staining

The SP method was used, and the first antibody was mouse-anti-human monoclonal CD68 antibody (DAKO, dilution 1:50). The operation procedure was according to the instructions of SP kit which was purchased from DAKO, Glostrup, Denmark. DAB was used for coloration. The dark brown granules in cytoplasm were taken as CD68 positive reaction. Negative mouse serum and PBS were respectively used to replace 1st antibody as negative control and blank control.

Identification of Kupffer cells

Among the cells which were anti-CD68 antibody positive, those in the blood space of cancerous tissues or the sinusoids of noncancerous tissues with a stellate or spindle shape were evaluated as Kupffer cells.

Determination of Kupffer cell numbers

The number of Kupffer cells was counted in five randomly selected visual fields under a microscope (×200) for each specimen. Then the average Kupffer cell number of each specimen was determined.

Statistical analysis

The data of Kupffer cells were expressed as mean ± standard deviation (Mean ± SD), and the differences in the values of different groups were analyzed by t test and F test. The criterion of significance was set at P<0.05.

RESULTS

Kupffer cells in HCC specimens

Kupffer cells were present in the cancerous tissues of 45 out
of 48 cases of HCCs (93.8%). The only 3 specimens in which no Kupffer cells were found were all poorly-differentiated HCCs. Kupffer cells were found in the para-cancerous tissues and adjacent normal liver tissues of all 48 HCCs.

**Number of Kupffer cells and histological grades**
The mean number of Kupffer cells in cancerous, para-cancerous and adjacent normal liver tissues was 12.7±6.8, 18.1±8.2 and 18.9±7.9 respectively. The number of Kupffer cells in cancerous tissues was significantly lower than that in para-cancerous tissues and adjacent normal liver tissues \( (P<0.05) \) (Table 1). The number of Kupffer cells in cancerous tissues of well, moderately and poorly differentiated HCCs was 18.4±6.2, 11.2±6.2 and 5.2±4.9, respectively (Table 2). The number of Kupffer cells in cancerous tissues significantly decreased as the histological grades decreased \( (P<0.05) \) (Figure 1).

### Table 1 Kupffer cell number in different tissue types

| Tissue type                  | n   | Kupffer cell number (x±s) |
|------------------------------|-----|--------------------------|
| Cancerous tissue             | 48  | 12.7±6.8                 |
| Para-cancerous tissue        | 48  | 18.1±8.2                 |
| Adjacent normal liver tissue | 48  | 18.9±7.9                 |

\( ^{a}P<0.05 \) vs. cancerous tissues, \( ^{b}P<0.05 \) vs. cancerous tissues.

### Table 2 Relationship between Kupffer cell number, tumor size, and differentiation degree

| Tumor size (diameter) | n   | Kupffer cell number (x±s) |
|-----------------------|-----|--------------------------|
| <3 cm                 | 12  | 17.4±4.8                 |
| 3-5 cm                | 17  | 12.5±5.3                 |
| >5 cm                 | 19  | 7.9±5.8                  |

| Differentiation degree  | n   | Kupffer cell number (x±s) |
|-------------------------|-----|--------------------------|
| Well- differentiated     | 11  | 18.4±6.2                 |
| Moderately- differentiated | 20 | 11.2±6.2                |
| Poorly- differentiated   | 17  | 5.2±4.9                  |

\( ^{a}P<0.05 \) vs. among three groups, \( ^{b}P<0.05 \) vs. among three groups.

**DISCUSSION**

Kupffer cells are important in the host defense mechanism including normal metabolism, phagocytosis, cytokine generation and anti-tumor effects. They are also involved in the pathogenesis of liver diseases such as viral hepatitis, alcoholic liver injury, chemically mediated liver injury, liver fibrosis, ischemia and reperfusion injury in liver transplantation, and hepatocyte regeneration\(^{[9-23]}\). Functional capability of Kupffer cells is considered to decrease when the liver is impaired. Usually, light-microscope is used to identify Kupffer cells by confirming the presence of lipofuscin and hemosiderin pigments in the cytoplasm. Moreover, Kupffer cells can be identified by monitoring their unique ultra-structures. For example, they have numerous vesicles, well-developed lamellipodia in cytoplasm and lysosomes in various sizes. In this study, we used anti-CD68, an anti-human macrophage antibody, to identify macrophages. CD68 was expressed not only in the residential macrophages such as Kupffer cells, but also in the migrating macrophages. In view of this fact, Kupffer cells cannot be identified merely by being anti-CD68 positive. Morphological observations are also required to distinguish between these two cell types. For example, migrating macrophages are usually oval and contain abundant cytoplasm, while Kupffer cells usually have spindle or stellate-shaped cytoplasm and partly adhere to the sinusoidal endothelial cells\(^{[24-29]}\).

The origin of Kupffer cells in normal liver tissues remains
unidentified. One hypothesis postulates that Kupffer cells are originated from the macrophages which have been present in the premordial liver at the embryonal stage. The other hypothesis proposes that monocytes which are originated from bone marrow arrive, settle in the sinusoids and then differentiate into Kupffer cells. There are two possible mechanisms of Kupffer cells existing in cancerous tissues of HCC. (1) Under the environmental condition similar to normal sinusoids, migrating macrophages in the blood space change into Kupffer cell-like cells. (2) Kupffer cells in normal liver tissue are maintained in the cancerous tissues. Our results showed that all the three poorly differentiated HCCs contained no Kupffer cell in cancerous tissues. Moreover, Kupffer cells in cancerous tissues of poorly differentiated HCCs were significantly less than those of well or moderately differentiated HCCs. In view of the fact that the morphology of poorly differentiated cancerous tissues is quite different from that of normal liver tissue, the former hypothesis may be more reasonable. We also found Kupffer cell number in para-cancerous and adjacent normal liver tissues had no statistical difference, probably due to the fact that para-cancerous tissues present in the blood space closer to the normal sinusoids. On the other hand, Kupffer cells were found to be activated in the pathogenesis of liver injuries such as early-stage fibrosis and fatty liver hepatitis. Under these conditions the sinusoid structures usually were not destroyed seriously and Kupffer cell number did not decrease significantly.

The mechanism responsible for the tumoricidal activities of Kupffer cells is not yet completely known. Kupffer cells may execute their anti-tumor effect via increasing the production of some cytotoxic molecules such as NO, TNF-α, and IFN-γ, which inhibit the growth of tumor by damaging cellular DNA and inducing apoptosis. When implanted into normal and cirrhotic rat livers, rat HCC cells grew much more progressively in cirrhotic livers than in normal livers. Meanwhile, Kupffer cells were decreased profoundly in cirrhotic livers, resulting in markedly impaired phagocytic activity. Furthermore, profound decrease production of Kupffer cell-related cytokines was found to in cirrhotic livers.

Previous studies also found that Kupffer cells might play an important role in controlling occurrence and progression of liver metastasis. The possible pathway of Kupffer cells against liver metastasis might be that tumor cells were apoptotic via the Fas-Fas ligand system induced by TNF-α released from Kupffer cells. When the liver is chemically injured, Kupffer cells release biologically active mediators that promote the pathogenic process. Though there is evidence that indicates Kupffer cells play a stimulatory role in liver regeneration, presently Kupffer cells are thought to have the potential to exert both stimulatory and inhibitory influences on hepatocyte proliferation.

Enhanced magnetic resonance imaging (MRI) has been used to detect hepatic tumors. The method utilizes selective take-up mechanism of superparamagnetic iron oxide (SPIO) or chondroitin sulfate iron colloid into the reticuloendothelial cells such as Kupffer cells of the liver. Our findings indicated that the number Kupffer cells in cancerous tissues decreased significantly as the tumor size increased and histological grade decreased. Therefore, the enhanced MRI which utilizes the enhanced mechanism of superparamagnetic iron oxide (SPIO) or chondroitin sulfate iron colloid into the reticuloendothelial cells can be useful in estimation of histological degree of HCC. Imai et al. proposed that SPIO-enhanced MRI reflect Kupffer cell number in HCCs and dysplastic nodules and be useful in assessing the histological grades of HCCs, especially poorly-differentiated and moderately-differentiated cases. Recently, Kitamura et al. reported 18 HCCs detected by color Doppler sonography had either a marked reduction in the number or absence of Kupffer cells. In conclusion, the present study has shown that Kupffer cells are important in preventing development of HCCs, and tumor metastases. They are also involved in the pathogenesis of chemically mediated liver injury and viral hepatitis. Further study on the biological characteristics and function of Kupffer cells will contribute to the early diagnosis of hepatic tumors and new treatment strategies.

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