A selective tropism of transfused oval cells for liver

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INTRODUCTION

Hepatic oval cells were first described by Opie in 1944 and named as oval cells first by Farber in 1956[12]. Oval cells could be seen at the early stage of hepatocarcinogenesis induced by chemicals in animal and in the liver of human suffering from chronic hepatitis, cirrhosis and other chronic liver diseases[3,31]. The emergence of oval cells was considered initially to be related to hepatocarcinogenesis. Oval cell was once believed to be a progenitor cell of carcinoma in liver[20,21]. Recently, more and more evidences showed that oval cell may be a potential stem cell in liver and it could differentiate into hepatocyte and epithelial cell of bile duct in certain conditions[12,13]. It is believed now that the potential stem cells existing in liver might play a role in the repair of damaged liver. However, when and how oval cells could be activated still remains unclear. Our aim in this study is to explore the biological behavior of hepatic oval cells after transfused into the circulation of rat for interpreting possible activating mechanisms of hepatic stem cells.

MATERIALS AND METHODS

Culture of oval cells

Hepatic oval cells were isolated from SD male rats and a cell line of OC3 was established by Dr. Xue in our group[14]. Oval cells were cultured under a routine condition (37 °C, 5% CO2). The cells were collected and suspended in a solution of RPMI-1640 (Gibco BRL) without serum on the day of transfusion experiment, on standby.

Establishment of animal model and transfusion of oval cells

SD female rats, weighing 100-150 g, were used for the establishment of an animal model of liver-damaging. The model was made by means of a 2-AAF/CCl4 program according to Petersen[31]. In the experimental group, 2-acetylaminofluorene (2-AAF, Sigma), 2.5 g·L-1 in earthnut oil, was administered to stomach of rats every day for 14 days. On the 7th day of 2-AAF administering, a LD50 dose of CCl4, was given by intraperitoneal injection. Then, the suspended oval cells (5x10⁶ cells per rat) were transfused into the circulation of the rats through caudal vein in 24 hours after CCl4 injection. In the control group, 1 ml earthnut oil per day was administered to stomach of rats instead of 2-AAF, and without CCl4 injection. The other treatments was the same as in the experimental group. The animals were sacrificed on the 7th day after transfusion of oval cells. The liver, kidney and spleen of the rats were picked out, respectively and frozen rapidly in liquid nitrogen. The frozen tissues were kept in -80 °C refrigerator.

Isolation of DNA

DNA was extracted from the frozen tissues of liver, kidney and spleen respectively according to the protocol of our laboratory. DNA samples were kept in -80 °C refrigerator.

Primer selection of sry gene

The primers selection was according to the DNA sequence of rat sry gene from GenBank Database (Accession No.: AJ222688): SryF17: 5'-catctgactctcggtgtgcaaa-3' , SryR16: 5' -atgctggattctgttgagcc-3'. The PCR product was 241 bp in length, corresponding to the sequence between 273-514 of rat sry gene.

PCR reactions

The DNA samples from liver, kidney and spleen in each experimental group were used as a template in PCR reactions. The reaction cocktails (containing 1 μg Template DNA, 0.125 mmol·L-1 dNTPs, 0.4 μmol·L-1 sry F17 primer, 0.4 μmol·L-1 sry R16 primer, 1×PCR buffer, 2.5 μmol·L-1 MgCl2, 1 U Taq-polymerase, add H2O to 50 μL of total volume) were run on GeneAmp® PCR System 9700 (AB) with a program combination of Prog. 1 (95 °C, 5 min), Prog. 2 (95 °C, 30 sec; 56 °C, 30 sec; 72 °C, 60 sec) and Prog. 3 (95 °C, 50 sec; 56 °C, 50 sec; 72 °C, 60 sec). The PCR products were
electrophoresed in 1.2 % agarose, stained with ethidium bromide and photographed.

In situ hybridization

In situ hybridization was carried out according to the protocol described by Zhao[15]. A DNA probe complementary to rat sry gene was labeled with digoxigenin by means of PCR reactions. The sections of liver from rats transfused with oval cells were selected for in situ hybridization assay. After deparaffin and rehydrate, the sections were fixed in 4 % paraformaldehyde again. The hybridization (2×SSC, 500 mL·L⁻¹ formamide, 1×Denhardt’s solution, 0.5 g·L⁻¹ dextran sulfate, 60 µg·L⁻¹ DIG-Probe) was carried out at 37 °C over night. The hybrids were then revealed by an alkaline phosphatase-conjugated anti-digoxigenin antibody and detected with the detection system of Boehringer Mannheim.

RESULTS

Sry gene was located in Y chromosome and used as a marker of transfused oval cells in female animal. The results of the cell-transplant experiment showed that the sry gene was detectable only in the liver but not in the spleen and the kidney of the rats treated by 2-AAF/CCl₄ program, and no signals could be detected in the control animals, neither liver nor spleen and kidney. The distribution of PCR signals of sry gene in experimental groups can be seen in Figure 1 and Table 1. On the section of in situ hybridization, a cluster of cells with sry gene marker could be seen in the parenchyma of the liver of a female rat undergoing oval cell-transplantation (Figure 2A). It was distinguished between sections in the negative and positive controls (Figure 2B and Figure 2C). This result meant that some exogenous cells had migrated into the parenchyma of the liver and settled there.

![Figure 1](image1.png)

**Figure 1** PCR signals of sry gene in experimental groups. (M. 100 bp DNA marker; 1. Liver(M): liver of male rat as a positive control; 2. Liver(E): liver of female rat in experimental group; 3. Spleen(E): spleen of female rat in experimental group; 4. Kidney(E): kidney of female rat in experimental group; 5. Liver(C): liver of female rat in control group; 6. Spleen(C): spleen of female rat in control group; 7. Kidney(C): kidney of female rat in control group; 8. Liver(F): liver of female rat negative control).

|       | Liver (M) | Liver (E) | Spleen (E) | Kidney (E) | Liver (C) | Spleen (C) | Kidney (C) | Liver (F) |
|-------|-----------|-----------|------------|------------|-----------|------------|------------|----------|
| Sry   | +         | +         | -          | -          | -         | -          | -          | -        |

**Table 1** Distribution of PCR signals of sry gene in experimental groups

Note: Liver(M): liver of male rat as a positive control; Liver(E): liver of female rat in experimental group; Spleen(E): spleen of female rat in experimental group; Kidney(E): kidney of female rat in experimental group; Liver(C): liver of female rat in control group; Spleen(C): spleen of female rat in control group; Kidney(C): kidney of female rat in control group; Liver(F): liver of female rat negative control.

DISCUSSION

As we know, liver has a powerful capacity of regeneration. In general, the injured liver can regenerate itself by self-replication of hepatocyte. So, it is believed that hepatocytes themselves are the functional stem cells of the liver[16]. Oval cell is now recognized as a potential stem cell existing in liver. Oval cell could differentiate toward hepatocyte and epithelial cell of bile duct[12,13,27-30]. So it was guessed that the emergence of oval cells might be relevant to the repair of...
damaged liver, and under special conditions the injured liver might produce and release some “signal molecules” which might play an important role in the activation of stem cell. In this study, an animal model of liver-damaging was established by a 2-AAF/CCl₄ program according to Petersen[11], the capacity of regeneration of hepatocyte was first impaired by 2-AAF and then the liver was damaged severely by CCl₄. In this status the damaged liver might produce a signal of “distress call” to initiate the activation of stem cell. Our results of cell transplantation showed that the sry gene was detectable only in the liver but not in the spleen and the kidney of the rats treated by 2-AAF/CCl₄ program, and no signals could be detected in the control animals, neither liver nor spleen and kidney. The results of in situ hybridization also showed that some exogenous cells had migrated into the parenchyma of the liver and settled there. It means that the transplused oval cells have a selective tropism for liver and the driver force might come from the injured liver. All evidences revealed that some “signal molecules” might exist in the circulation of the rats treated by 2-AAF/CCl₄ and the “signal molecules” might be produced and released from the damaged liver. The “signal molecules” might play an important role in the initiation of the activation of stem cell. Further identifying and isolation of these “signal molecules” would be significative for achieving activation and directional inducement of hepatic stem cells.

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