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• BASIC RESEARCH •

A selective tropism of transfused oval cells for liver

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Abstract

AIM: To explore the biological behaviors of hepatic oval cells after transfused into the circulation of experimental animals.

METHODS: Oval cells from male SD rat were transfused into the circulation of a female rat which were treated by a 2-AAF/CCl_4 program, through caudal vein. Sex-determining gene *sry* which located on Y chromosome was examined by PCR and in situ hybridization technique in liver, kidney and spleen of the experimental animals, respectively.

RESULTS: The results of the cell-transplant experiment showed that the *sry* gene was detectable only in the liver but not in spleen and kidney of the experimental rats, and no signals could be detected in the control animals. It can be also morphologically proved that some exogenous cells had migrated into the parenchyma of the liver and settled there.

CONCLUSION: The result means that there are exogenous cells located in the liver of the experimental animal and the localization is specific to the liver. This indicates that some "signal molecules" must exist in the circulation of the rats treated by 2-AAF/CCl_4. These "signal molecules" might play an important role in specific localization and differentiation of transfused oval cells.

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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[1,2]. 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[3-8]. 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[9-11]. 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 cultivated under a routine condition (37 ℃, 5 % CO_2). 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/CCl_4 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 everyday for 14 days. On the 7th day of 2-AAF administering, a Ld50 dose of CCl_4 was given by intraperitoneal injection. Then, the suspended oval cells (5×10^6 cells per rat) were transfused into the circulation of the rats through caudal vein in 24 hours after CCl_4 injection. In the control group, 1 ml earthnut oil per day was administered to stomach of rats instead of 2-AAF, and without CCl_4 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 ℃ 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 ℃ refrigerator.

Primers selection of sry gene

The primers selection was according to the DNA sequence of rat *sry* gene from GenBank Database (Accession No.: AJ222688): *Sry*F17: 5' -catctctgacttcctggttgcaa-3' , *Sry*R16: 5' -atgctgggattctgttgagcc-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 m mol· L^{-1} dNTPs, 0.4 μmol· L^{-1} sry F17 primer, 0.4 μmol· L^{-1} sry R16 primer, 1×PCR buffer, 2.5 m mol· L^{-1} $MgCl_2$, 1 U Taq-polymerase, add H_2O to 50 μL of total volume) were run on GeneAmp® PCR System 9 600 (AB) with a program combination of Prog. 1 (95 ℃, 5 min), Prog. 2 (95 ℃, 50 sec; 56 ℃, 50 sec; 72 ℃, 60 sec; 30 cycles) and Prog. 3 (95 ℃, 50 sec; 56 ℃, 50 sec; 72 ℃, 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 % paraformaldhyde 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 ℃ 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/CCl_4 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 meaned that some exogenous cells had migrated into the parenchyma of the liver and settled there.

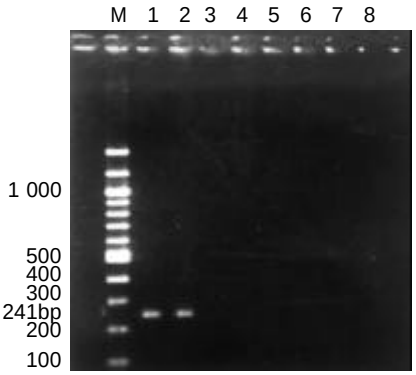

Figure 1 PCR signals of *sry* gene in experimental groups. (M. 100 bp DNA marker; 1. Liver(M): liver of male rat as a positive control; 2. Liver(E): liver of female rat in experimental group; 3. Spleen(E): spleen of female rat in experimental group; 4. Kidney(E): kidney of female rat in experimental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

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

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

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.

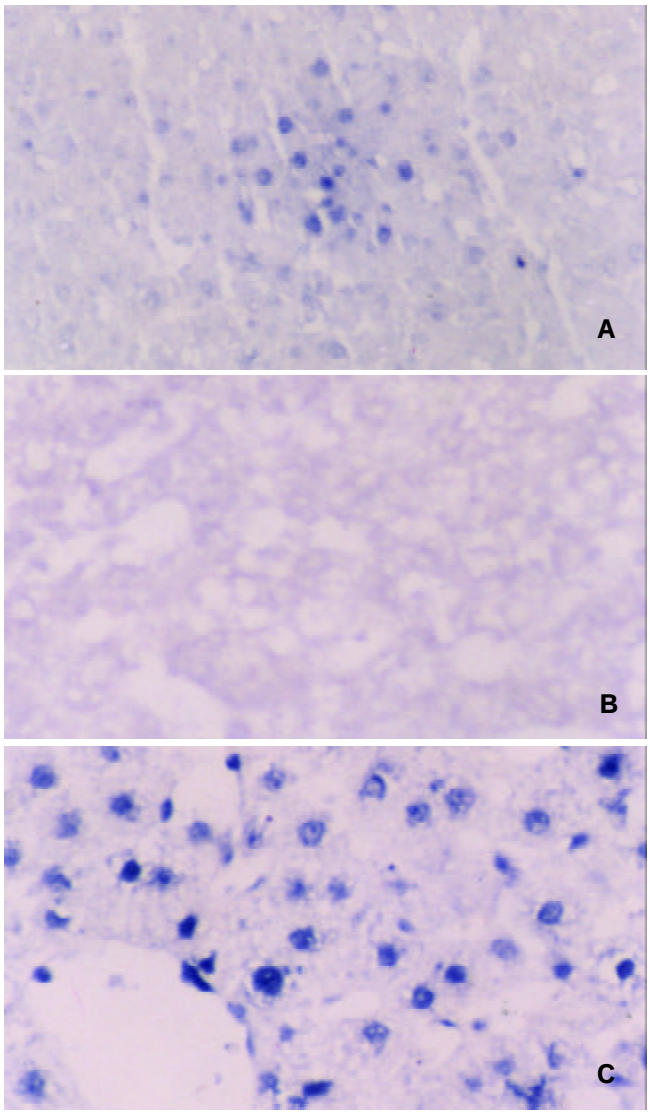

Figure 2 In situ hybridization assay of *sry* gene in liver. A. Experimental group, Liver of female rat undergoing oval cell-transplanting. A cluster of cells with *sry* gene marker could be seen in the parenchyma of liver. B. Negative control group, liver of normal female rat. No hybridization signals could be seen. C. Positive control group, liver of normal male rat. Each cell with distinct hybridization signals.

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. But they cannot be seen in normal status. The emergence of oval cells occurs only in a special status in which the liver is damaged severely and the capacity of regeneration of hepatocyte is restrained[17-20]. At the past, oval cell was believed as a progenitor cell of carcinoma in liver[9-11,21], because it was often seen at the early stage of hepatocarcinogenesis induced by chemicals[22-26]. Recently, more and more evidences showed that 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/CCl_4 program according to Petersen[31], the capacity of regeneration of hepatocyte was first impaired by 2-AAF and then the liver was damaged severely by CCl_4. 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/CCl_4 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 transfused 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/CCl_4 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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