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Transferrin Receptors in the Liver Cell Membrane of Carcinogen (3-methyl-4-dimethyl-aminoazobenzene) Treated Rat*

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ABSTRACT

To investigate the alteration of transferrin receptor (TfR) in the proliferating or transformed liver cells, ^{125}I-transferrin binding experiment was carried out in the isolated parenchymal cells (PC) or nonparenchymal cells (NPC) from normal regenerated rat liver after partial hepatectomy and from the liver of 3-methyl-4-dimethyl-aminoazobenzene (3-Me-DAB) treated rat.

With the administration of 3-Me-DAB for 8 weeks, the liver tissue showed marked morphologic changes of oval cell proliferation, regenerations of hepatocytes, and atypical proliferations of bile ducts, but these changes were little affected by partial hepatectomy.

Transferrin binding values in PC or NPC homogenate from the regenerated liver of normal rat, were increased by 3rd day and diminished to control level at 7th day after partial hepatectomy. With the treatement of 3-Me-DAB for 8 weeks, transferrin binding sites in homogenates were higher than those of normal rat liver and increased by 7th day after partial hepatectomy.

Transferrin binding sites (Bmax) in the cell membrane of NPC were higher than those of PC of normal rat liver, but there was no significant difference in Kd values between both groups (5.05, 6.3 nM).

In the normal regenerated rat liver, transferrin binding sites in the PC or NPC plasma membrane, were increased by 3rd day and diminished to control level at 7th day after partial hepatectomy.

With 3-Me-DAB tratment, transferrin binding sites in both liver NPC and PC plasma membrane were increased about 3 folds, compared to those in each plasma membrane of normal rat liver. And after partial hepatectomy of 3-Me-DAB trated rat, transferrin binding sites were increased by the 3rd day in the NPC plasma membrane but increased by the 7th day in the PC plasma membrane.

In the transferrin binding sites of the PC or NPC plasma membrane of 3-Me-DAB treated liver, two kinds of Kd values (3.1~4.7 nM, 25.4~54.1 nM) were detected.

The present results suggest that 1) TfRs are distributed in the liver PC as well as NPC; 2) Increased TfRs in PC or NPC plasma membrane of normal regenerated liver after partial hepatectomy and 3-Me-DAB treated rat liver, may be due to increased intracellular synthesis; 3) Increased TfRs in normal regenerated liver after partial hepatectomy might be related to the expression of a single type of high affinity site (Kd, 3.1~7.5 nM), but in 3-Me-DAB treated rat liver might be related to the expression of high and low affinity types of receptors (Kd, 25.4~54.1 nM).

Key Words: Transferrin receptor, 3-Methyl-4-dimethyl-aminoazobenzene, Regenerated liver, Liver parenchymal cell, Liver nonparenchymal cell

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INTRODUCTION

All living systems require iron for growth and survival. In vertebrates, iron transport is mediated by the transferrin protein, while cellular iron is stored chiefly by ferritin. Transferrin receptors (TfR) in cell surface can bind with specifically and facilitate entry of iron-transferrin complex (ferrotransferrin) into cells via a process known as receptor mediated endocytosis (May and Cuatrecases 1985; Bothwell *et al.*, 1958; Trowbridge and Omary 1981; Sutherland *et al.*, 1981; Fraizer *et al.*, 1981; Seligman 1983; Young and Bomford 1983).

TfRs are rich in most rapidly proliferating normal and transformed cells, and drastically diminishes when cells are induced to terminally differentiate. Thus the TfR is appreciated as a specific surface marker for rapidly growing cells and its expression is closely linked to the proliferation status of the cell (Ursula *et al.*, 1988; Girons and Davis 1989; Keer *et al.*, 1990; Ciechanover *et al.*, 1983).

Extensive resection of normal liver is compatible with return to full health because of the capacity of the remnant to regenerate. In the rat, for example, following about 70% hepatectomy, DNA synthesis is peak at 22~24 hours and normal mass is restored within 7~10 days (Pezzino *et al.*, 1967; Bucher 1967). After partial hepatectomy, insulin and glucagon receptors are found to be changed and TfRs in the cell surface are increased (Akiko *et al.*, 1989; Akiko and Akamatsu 1989).

Futhermore the rate of endocytic cycle of TfRs in the transformed cells or mutant TfRs are different to those in the normal cell line (Sorokin *et al.*, 1988; Jing *et al.*, 1990; Rothenberger *et al.*, 1987), and TfRs in the HL-60 premyelocytic leukemia cells are phosphorylated and unphosphorylated type, while those in a variant of this cell line are only phosphorylated type (Ishiguro *et al.*, 1992).

3-Methyl-4-dimethyaminoazobenzene (3-Me-DAB), a potent azo-dye carcinogen can induce oval cell proliferation, hyperplastic nodule and cholangiofibrosis in the liver (Goldfarb, 1973; Tsao and Grisham, 1987; Park *et al.*, 1991). Although it is generally believed that hyperplastic nodule and cholangiofibrosis may transit to hepatoma and cholangiocarcinoma, functional or mechanical change of TfRs in the transformed cell by these carcinogen has not been defined.

In the TfRs of liver cell, Vogel *et al.*, (1987) had reported that it was distributed 3 to 4 times more in the hepatocyte (parenchymal cell, PC) than in the nonparenchymal cell (NPC). However, Soda and Tavassoli (1984), and Kishimoto and Tavassoli (1985) recently reported that they could demonstrate neither binding of transferrin coated minibeads nor binding of radiolabeled transferrin to parenchymal cells in rat liver. They concluded that only hepatic endothelial cells expressed TfRs and iron might be first taken up by liver endothelium and then transmitted to parenchymal cells.

In the present study, we carried out transferrin binding experiment to investigate any change of TfRs in the isolated PC or NPC from normal regenerated rat liver after partial hepatectomy or from the liver of 3-Me-DAB treated rat.

MATERIALS AND METHODS

Partial hepatectomy and carcinogen treatement

Male 6-weeks-old Sprague-Dawley rats, weighing 120 to 150 g were used in this study. Partial hepatectomy (about 70%) was performed using the method described by Higgins and Anderson (1931) and Lee *et al.* (1975). This involved removal of the median and left lateral lobe. Liver cells were isolated from nonoperated normal rat and from operated groups of 1, 3 or 7 days after partial hepatectomy.

In the carcinogen treatement, Sprague-Dawley rats (100~150 g) were fed a solid diet containing 0.08% 3-Me-DAB dissolved in corn oil for 8 weeks, and liver cells were isolated from these nonoperated groups and from the groups of 1, 3 or 7 days after partial hepatectomy of 3-Me-DAB fed rat.

Isolation and fractionation of liver cell

Liver cell suspensions were prepared by a collagenase perfusion method (Seglen, 1976). Briefly, 0.3 ml of heparin (1000 IU/ml) was injected into the tail vein and the liver was perfused

through the cannulated portal vein with warm Ca-free buffer at the rate of 870 ml/hr. The buffer was contained 140 mM NaCl, 6 mM KCl, 6 mM NaOH, 11 mM glucose, and 10 mM HEPES, pH 7.5. The liver was isolated and perfusion was subsequently continued with a 15 ml of collagenase buffer, which was contained 0.1% collagenase (type IV. Sigma), 0.004% DNase, 1.5 mg/ml bovine serum albumin (BSA) and 2 mM $CaCl_2$ (Table 1). To maintain the optimal temperature of 37℃ for collagenase activity, the buffer was kept in a water bath at 42℃.

After perfusion of collagenase buffer to elute liver cells, the remnant liver tissue was then stirred in collagenase buffer at 37℃ for 15 min. All eluted liver cells were filtered with 3 layers of cheeze cloth. The filtered liver cells suspension was centrifuged 2 times at 50×g for 5 min to remove cellular debris. The cell pellet was then resuspended with small volume of Dulbecco's phosphate-buffered saline (PBS) (Table 1).

Fractionation of this cell suspension was done by the method of Vogel *et al.* (1987). Briefly, the isolated liver cell suspension was layered on 20%/30%/70% buffered percoll solution. After centrifugation for 40 min at 4500 rpm (Sorvall, RC-5B), NPCs were recovered from the upper layer of 20% percoll solution, and PCs were collected from the interface layer of 30% and 70% percoll solution. Each fraction was gently collected and washed 2 times with Dulbecco's PBS. The microscopic pro-

Table 1. Composition of buffers used for liver cell isolation

	Ca-free buffer	Collagenase buffer	D-PBS*
NaCl	8,300	4,000	8,000
KCl	500	500	200
1 M NaOH (ml)	6	66	–
HEPES	2,400	24,000	–
KH_2PO_4	–	–	200
Na_2HPO_4	–	–	2,160
$CaCl_2 \cdot 2H_2O$	–	700	–
Collagenase	–	1,000	–
DNase	–	40	–
BSA	–	15,000	–
Glucose (gm)	1.9	1.9	–
pH	7.5	7.6	7.4

Salt concentrations except glucose and NaOH are given in milligrams per 1,000 ml of final solution. The strongly buffered solutions are designed to withstand continuous acidification by the liver cells, and therefore have a high initial pH.

*Dulbecco's phosphate buffered saline.

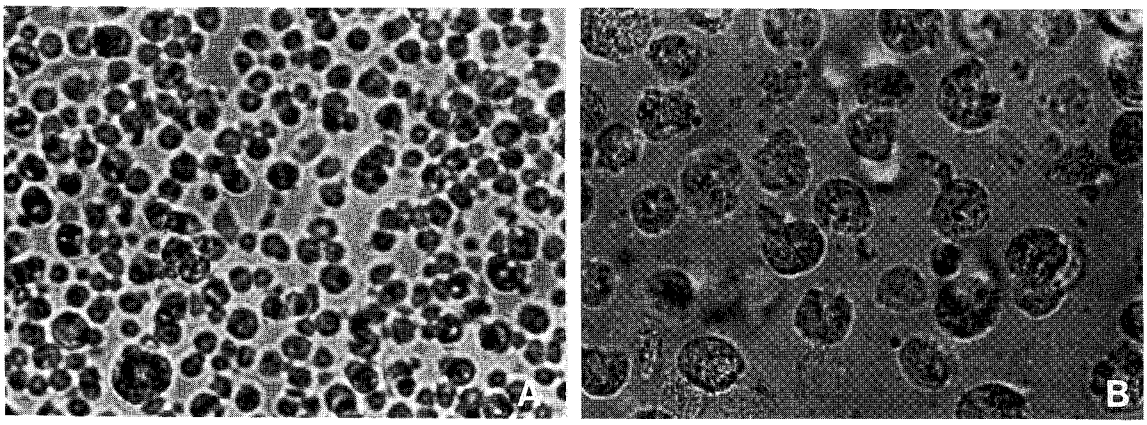

Fig. 1. Hepatic parenchymal and nonparenchymal cells purified by discontinuous percoll gradient centrifugation. An initial isolated liver cell suspension was layered on 20%/30%/70% buffered percoll solution. After centrifugation for 40 minutes at 4,500 rpm, nonparenchymal cells (A, × 250) were recovered from the upper layer of 20% percoll, and parenchymal cells (B, × 250, inverted microscope) were collected from the upper layer of 70% percoll.

portion of different cell types in these fractions (inverted microscope, American Optical Co) are given in Fig. 1.

Preparation of liver cell plasma membrane

To evaluate the alteration of cellular receptor, plasma membrane was prepared from liver PC or NPC by the method of Molitoris and Simon (1985) with some modification. The each liver cells fraction was suspended in Tris buffer (300 mM Mannitol, 5 mM EGTA, 0.1 mM phenylmethylsulfonylfluoride, 18 mM Tris-HCl, pH 7.4), homogenized with Polytron homogenizer (Kinematica Co.) and motor driven glass teflon pestle (Wheaton, 10 ml) for 10 strokes. This homogenate was used to detect total cellular receptor and an aliquot of homogenate was centrifuged at 1000 rpm for 15 min using Sorvall SS-34 rotor. The resulting supernatant was carefully overlayered on a discontinuous sucrose gradient (0.6 M and 1.2 M sucrose) solution and centrifuged at 30,000 rpm for 60 min (Sorvall OTD-75B ultracentrifuge, AH-650 rotor). The interface layer of 0.6 and 1.2 M sucrose solution was harvested and washed in Tris buffer (30,000 rpm for 30 min). The pellet, plasma membrane fraction, was resuspended in 0.5 to 1 ml of Tris buffer and stored at −70℃. The entire procedure was performed at 4℃ and protein concentration was determined by the method of Bradford (1976) with bovine serum albumin as a standard.

Histological examination

Liver tissues removed promptly from the 3-Me-DAB fed rats for 8 weeks with or whithout partial hepatectomy, were fixed in 10% formalin solution. Sections, 4 μm thick were prepared with a paraffin block and washed three times in phosphate buffer saline. Serial sections of liver were stained with hematoxylin and eosin for histological examination.

Transferrin receptor assay

^{125}I-transferrin binding was induced in the solution contained 100 mM NaCl, 0.073 mg/ml BSA, 50 mM HEPES, pH 7.4. After incubation of each purified cell membrane (50 μg) or homogenate fraction (200 μg) for 30 min at 37℃ with ^{125}I-transferrin (0.5 to 100nM), the reaction mixture was added with 2 ml of 50 mM HEPES buffer, and centrifuged at 150,000 × g for 20 min (Sorvall OTD-75B, AH-650). The pellet was washed 2 times with cold HEPES buffer. The radioactivity of ^{125}I-transferrin associated with the membrane pellet, was measured with a γ-scintillation counter (Packcard, 500-C). Specific binding site was calculated from the difference in values observed in the absence (total binding) and presence of 500 folds of unlabeled transferrin (nonspecific binding). Scatchard plots (Scatchard 1949) were used to estimate the number of maximum transferrin binding sites (Bmax) and affinity constant (1/Kd) of the TfR.

RESULTS

Histological findings in the liver of 3-Me-DAB treated rat

In the liver of rat fed the continuous 0.08% 3-Me-DAB for 8 weeks, there were marked morphologic changes of scattered necrotic areas associated with lymphocytic infiltration, proliferations of bile ducts, advanced bridging necrosis, hyperplastic nodule of hepatocytes and atypical proliferation of bile ducts. Adenocarcinoma was appeared and oval cells were infiltrated around the necrotic foci in the liver of 7th day after partial hepatectomy. But there were little difference between the gross morphologic changes of liver of 3-Me-DAB treated rat and those of liver with partial hepatectomy of 3-Me-DAB treated rat(Fig. 2).

Transferrin binding to liver cell homogenate

To measure total cellular TfR numbers in normal regenerated liver or in the liver of carcinogen trated rat, transferrin binding assay with homogenate fraction of isolated liver cells was carried out (Table 2). In the normal rat liver, transferrin binding values of liver NPC were 40~70% higher than those of liver PC (NPC: 122.85, 244.21 fmol/mg protein, PC: 85.93, 104.4 fmol/mg protein at 5 or 100 nM transferrin respectively). Transferrin binding sites of NPC in regenerated liver at 1st or 3rd day after partial hepatectomy were increased to 131 and 324 or 210 and 370 fmol /mg protein in the presence of 5 and 100 nM transferrin respectively. These results suggest that

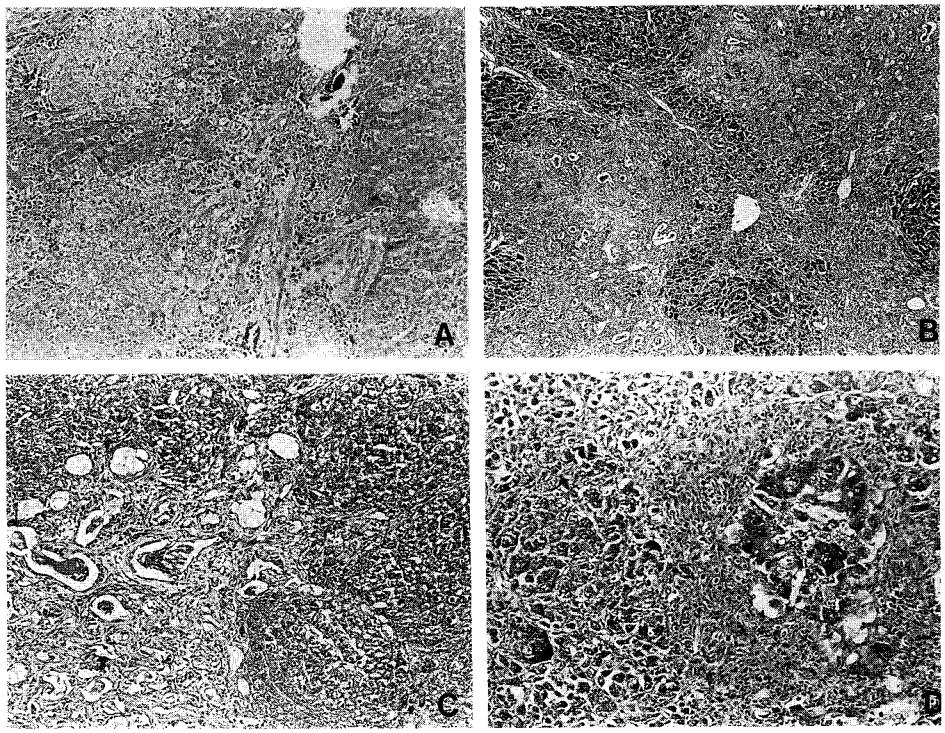

Fig 2. Morphologic changes of livers of rat fed 3-Me-DAB for 8 weeks with or without patial hepatectomy (PH). (A) Liver of rat treated with 3-Me-DAB. There are scattered necrotic areas associated with lymphocytic infiltration and proliferation of bile ducts (× 100). (B) Liver of 1 day after PH of 3-Me-DAB treated rat, advanced bridging necrosis and atypical proliferation of bile ducts(× 40). (C) Liver of 3 days after PH of 3-Me-DAB treated rat, some nodular regenerative changes of hepatocytes, irregular shaped bile ducts and moderate proliferated oval cells (× 100). (D) Liver of 7 days after PH of 3-Me-DAB treated rat, marked proliferation of oval cells around the necrotic foci(× 200).

Table 2. Specific ^{125}I-transferrin binding to homogenate of parenchymal and nonparenchymal cell of normal and 3-Me-DAB treated rat liver

($\times 10^{-9}$ M)	NPC		PC	
	5	100	5	100
Normal rat (fmol/mg protein)				
Cont	122.85±18.52	244.21±28.59	85.93± 9.75	140.40±18.86
R 1	131.36± 4.88	323.93± 6.43	136.06± 7.25	171.47±12.32
R 3	210.19±16.45	370.08±19.68	178.88±12.77	329.43±19.21
R 7	100.65±10.65	169.45±24.28	77.04± 8.11	134.46±10.52
3-Me-DAB treated rat (fmol/mg protein)				
Cont	216.68±19.32	325.45±24.89	154.80±10.01	255.88±31.56
R 1	189.89± 9.83	440.45±43.50	175.24± 7.22	274.57±20.00
R 3	285.95±20.18	549.89±72.00	174.20±10.44	324.54±22.43
R 7	300.64±17.85	721.16±53.50	243.00±21.33	490.39±48.79

Each value represents mean ± SE obtained from 4 binding experiments at 5 or 100 nM ^{125}I-transferrin in reaction medium. NPC and PC: liver nonparenchymal and parenchymal cell. Cont, R 1, R 3, R 7: Control and regenerated liver for 1, 3 and 7 days after partial hepatectomy.

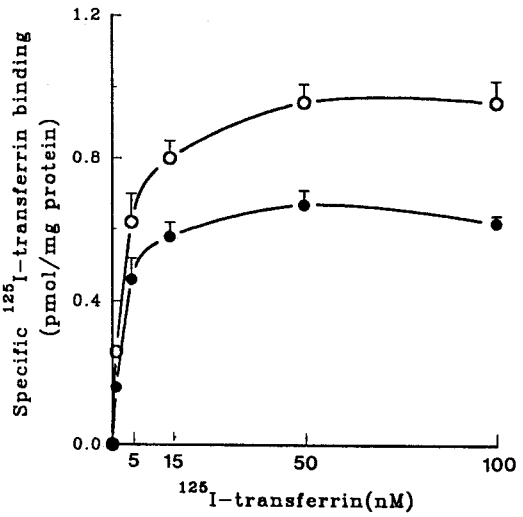

Fig. 3. Quantitation of transferrin binding sites in liver parenchymal and nonparenchymal cell membrane of normal rat.
Membrane (50 μg protein/tube) was incubated at 37℃ with indicated concentration of ^{125}I-transferrin in reaction mixture. Aliquots were removed after 30 min incubation, and centrifuged to remove free form of ^{125}I-transferrin. The amount of specifically bound radioligand was determined from the difference of transferrin binding value between in the absence (total binding) and presence (nonspecific binding) of 500 fold unlabeled transferrin. Each point represents the mean ± SE of 4 experiments. Transferrin binding is almost saturated at 15 nM of ^{125}I-transferrin.
●−●: specific biniding to parenchymal cell membrane, ○−○: specific binding to non-parenchymal cell membrane.

synthesis of TfR was increased in regenerated liver cells. However in the liver cells of 7th day after partial hepatectomy, transferrin binding sites were reduced to normal value. The patterns in the change of these transferrin binding sites in PC were similar to those in NPC.

In the liver cells of 3-Me-DAB treated rat, transferrin binding sites were increased about 33 ~76% in NPC and about 80% in PC (NPC: 216.68, 325.45 fmol/mg protein, PC: 154.8, 255.88 fmol/mg protein at 5 or 100 nM transferrin respectively), compared to those in corresponding cell fraction of normal rat liver. Therefore we guess that by 3-Me-DAB administration the synthesis of TfR was increased.

With partial hepatectomy of 3-Me-DAB treated rat, specific transferrin binding sites were further increased in both cells fractions, and it was increased about 40~120% in the liver of 7th day after partial hepatectomy compared to those values in nonoperated liver of 3-Me-DAB treated rat.

TfR in normal liver cell membrane

To investigate the distribution of TfR to the cell membrane of PC or NPC of normal rat liver, transferrin binding experiments were carried out with plasma membrane of each cell fraction, and the Kd and Bmax were calculated by Scatchard analysis (Fig. 3, Table 3). As shown in Fig. 3, transferrin binding values were increased as the increase of ^{125}I-transferrin concentration in the cell membrane of both cell fractions. These binding sites were higher approximately 40% in the cell membrane of NPC than PC. By Scatchard analysis of the data for transferrin binding to both cell membranes, Kd values were 5.0 and 6.3 nM, in NPC and PC membrane respectively (Table 3). But maximum binding sites (Bmax) in NPC membrane were 0.99 pmol/mg protein, whereas those in PC membrane were 0.7 pmol/mg protein. These results suggest that TfRs are more distributed in the NPC membrane than in the PC membrane with one kind of high affinity site.

TfR in regenerated liver cell membrane by partial hepatectomy

Changes of transferrin binding sites measured from PC or NPC membrane in regenerated liver after partial hartial hepatectomy are given in Fig. 4 and Table 3. Specific binding to NPC or PC membrane was concentration-dependent, and reached a plateau state at the concentration of 15 nM of ^{125}I-transferrin. These patterns were almost same as those of nonoperated normal rat liver cell membranes. Specific transferrin binding values were increased about 15~30% at 1st day, to a peak at 3rd day (increased about 50%) and fallen to normal value at 7th day after partial hepatectomy. In the Scatchard analysis of these transferrin binding values, the apparent binding affinities (Kd) of all membrane fractions were in

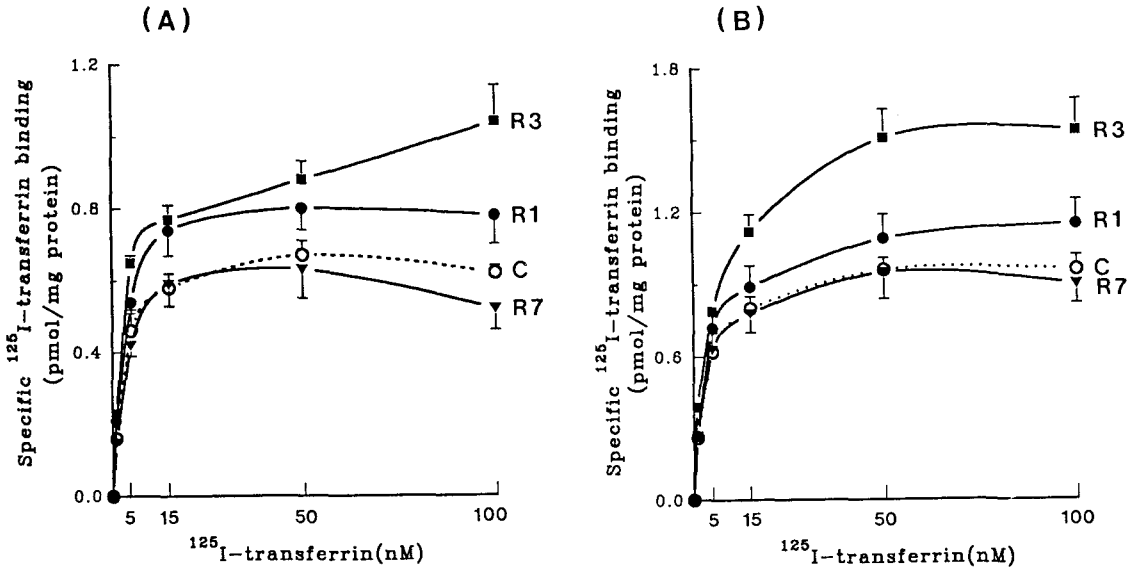

Fig. 4. Specific binding of ^{125}I-transferrin in liver cell membrane of normal rat with or whithout partial hepatectomy. (A) Specific transferrin binding in parenchymal cell membrane. (B) Specific transferrin binding in nonparenchymal cell membrane
Details are the same as in Fig. 3.
C: control liver. R 1, R 3 and R 7: regenerated liver for 1, 3 and 7 days after partial hepatectomy.

Table 3. Scatchard analysis of transferrin binding sites in parenchyml and nonparenchymal cell membrane of normal rat liver with or without partial hepatectomy

	Regression equation	Kd (nM)	Bmax (pmol/mg protein)
NPC			
Cont	Y=0.193−0.198 X (r=−0.99)	5.05	0.99
R 1	Y=0.202−0.177 X (r=−0.99)	5.64	1.14
R 3	Y=0.302−0.285 X (r=−0.96)	4.88	1.47
R 7	Y=0.200−0.212 X (r=−0.99)	4.71	0.95
PC			
Cont	Y=0.110−0.159 X (r=−0.95)	6.30	0.70
R 1	Y=0.120−0.134 X (r=−0.92)	7.47	0.90
R 3	Y=0.169−0.169 X (r=−0.99)	5.88	1.10
R 7	Y=0.107−0.158 X (r=−0.96)	6.30	0.68

Kd and Bmax were calculated from Scatchard analysis from the data in Fig. 4. and Fig. 5. Legends are the same as in Table 2.

the range of 4.71~7.47 nM (Table 3). Transferrin binding sites (Bmax) in the cell membrane of liver at 3rd day after partial hepatectomy, were increased about 50% (NPC: 1.47, PC: 1.10 pmol/mg protein) compared to those in nonoperated normal rat liver cell membrane, but less in the PC membrane than those in NPC membrane. There was little change in the ratio of TfR distribution

of liver PC to NPC membrane by partial hepatectomy.

Effct of 3-Me-DAB on the TfR in PC or NPC membrane

Specific transferrin binding sites in liver NPC and PC membrane of 3-Me-DAB treated rat at various concentrations of ^{125}I-transferrin are shown in Fig. 5. In contrast to the cell membrane of normal rat liver, transferrin binding sites were not saturated at 15 nM and still increased at 100 nM of ^{125}I-transferrin in both cell membrane fractions. Thus in the Scatchard analysis, linear regression equations showed 2 kinds of slopes or Kd values. One was a high affinity site (Kd: 3.3 nM) that is in the range of those in the normal rat liver, the other was a low affinity site (Kd: 39.5 or 33.1 nM) that is not in the normal rat liver (Fig. 6, Table 4). Bmax of high affinity sites in the liver PC or NPC membrane of 3-Me-DAB treated rat, were increased about 30～40% (PC: 0.93, NPC: 1.38 pmol/mg protein), compared to those in each cell membrane of normal rat liver. And total binding sites in PC or NPC membrane were about 3 fold (PC: 1.96, NPC: 2.97 pmol/mg protein) of

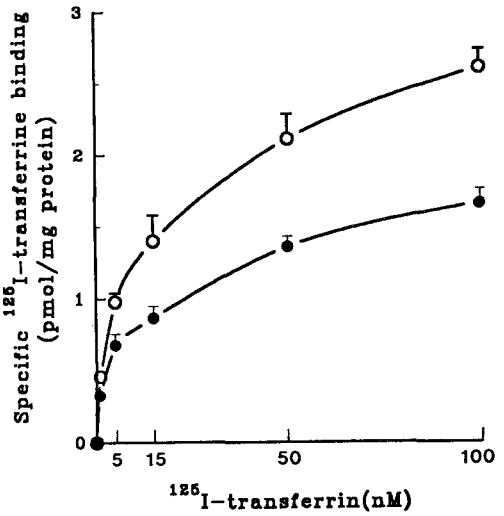

Fig. 5. Specific binding of ^{125}I-transferrin in parenchymal and non-parenchymal cell membrane of liver of 3-Me-DAB treated rat.
Details are the same as in Fig. 3.
Transferrin binding is not saturated at 15 nM of ^{125}I-transferrin in reaction mixture in contrast to that in normal rat liver cell membrane.
●—●: specific binding to parenchymal cell membrane, ○—○: specific binding to nonparenchymal cell membrane.

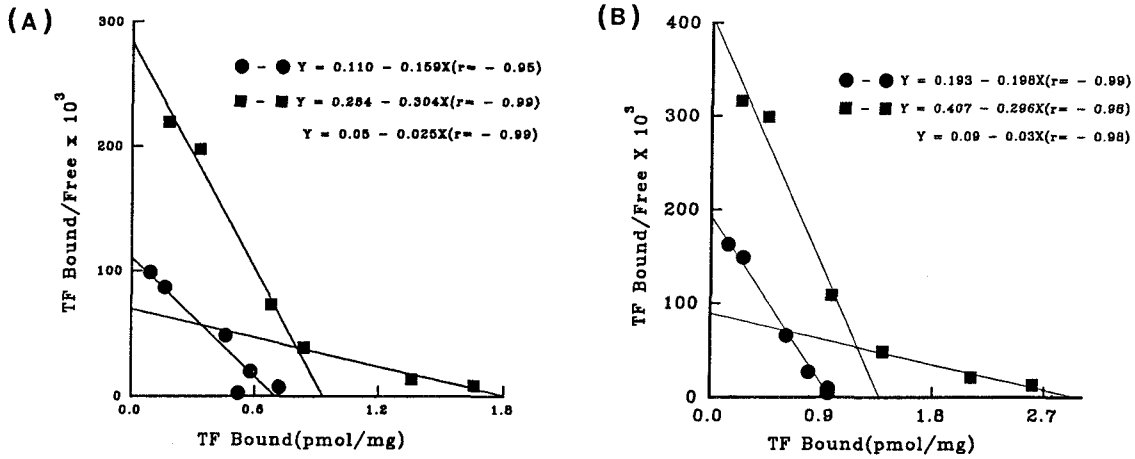

Fig. 6. Scatchard analysis of the specific binding of transferrin to the liver parenchymal cell (PC) and nonparenchymal cell (NPC) membrane of normal rat and 3-Me-DAB treated rat. Data of specific transferrin binding sites are from Fig. 3 and Fig. 5. (A) The receptor number (Bmax) for the PC plasma membrane in normal rat liver (●), calculated from the intercept of the abscissa, indicates 0.7 pmol/mg protein with affinity constant (Kd), calculated from the slope of the regression line, of 6.3 nM. But in 3-Me-DAB treated rat liver (■), there were two kinds of Kd; 3.28 and 39.5 nM. (B) Bmax for the NPC plasma membrane in normal rat liver (●) indicates 0.99 pmol/mg protein, with Kd of 5.05 nM. But 3-Me-DAB treated rat liver (■), there were two kinds of Kd; 3.38 and 33.11 nM.

Table 4. Scatchard analysis of transferrin binding sites in parenchymal and nonparenchymal cell membrane of 3-Me-DAB treated rat liver

	Regression equation	Kd (nM)	Bmax (pmol/mg protein)
From binding sites at 0.5−5 nM ^{125}I-transferrin			
NPC			
Cont	Y=0.407−0.296 X (r=−0.98)	3.38	1.38
R 1	Y=0.406−0.310 X (r=−0.97)	3.23	1.31
R 3	Y=0.437−0.225 X (r=−0.99)	4.43	1.94
R 7	Y=0.446−0.323 X (r=−0.94)	3.10	1.38
PC			
Cont	Y=0.284−0.304 X (r=−0.99)	3.28	0.93
R 1	Y=0.237−0.235 X (r=−0.99)	4.25	1.01
R 3	Y=0.342−0.252 X (r=−0.98)	4.38	1.46
R 7	Y=0.332−0.212 X (r=−0.98)	4.70	1.57
From binding sites at 15−100 nM ^{125}I-transferrin			
NPC			
Cont	Y=0.090−0.030 X (r=−0.98)	33.11	2.97
R 1	Y=0.108−0.031 X (r=−0.97)	31.85	3.44
R 3	Y=0.150−0.039 X (r=−0.99)	25.37	3.81
R 7	Y=0.099−0.035 X (r=−0.99)	28.88	2.85
PC			
Cont	Y=0.050−0.025 X (r=−0.99)	39.50	1.96
R 1	Y=0.064−0.028 X (r=−0.99)	35.84	2.28
R 3	Y=0.054−0.018 X (r=−0.94)	54.06	2.92
R 7	Y=0.074−0.025 X (r=−0.98)	39.70	2.95

Kd and Bmax were calculated from Scatchard analysis of data obtained from 4 binding experiments. Legends are the same as in Table 2.

those in each cell membrane of normal rat liver (Table 4).

In the liver with partial hepatectomy of 3-Me-DAB treated rat, the apparent Kd values from binding experiment at 0.5~5 nM ^{125}I-transferrin (high affinity site) were in the range of 3.1 to 4.7 nM, those from binding experiment at 15~100 nM ^{125}I-transferrin (low affinity site) were in the range of 25.4 to 54.1 nM. Both types of transferrin binding sites in liver NPC membrane of 3-Me-DAB treated rat, were increased by 3rd day and reduced to control level at 7th day after partial hepatectomy. But those in PC membrane were increased continuously by 7th day after partial hepatectomy. These results suggest that the enhanced effects of 3-Me-DAB on surface TfR concentration may be resulted from an increased high affinity sites (Kd: 3.1~7.4 nM) and appearance of other type of affinity sites (Kd: 25.4~54.1 nM).

DISCUSSION

Exposure to some drugs such as diethyl-nitrosamine, 2-acetylaminoflurane or 3-Me-DAB, results in a series of atypical proliferation, oval cell proliferation, hyperplastic nodule and cholangiofibrosis in the liver that occur prior to the development of hepatocellular carcinoma (Goldfarb, 1973; Tsao and Grisham, 1987; Park *et al.*, 1991). The results of this study, showing marked morphologic changes such as oval cell proliferation, regenerative hyperplastic nodule, and bile duct proliferation by 3-Me-DAB were similar to those of other investigators.

It is not yet determined which cells of the liver express the receptor of transferrin. Kishimoto and Tavassoli (1985) and Soda and Tavassoli (1984) reported that they could not demonstrate binding of transferrin to parenchymal cells in rat liver, suggested that TfRs were expressed only by the NPC, especially endothelial cells of rat liver. Thus iron might be first processed by the endothelial cell and then transported to other tissue cells. However, Vogel *et al.* (1987) recently reported that transferrin bound to receptors on PC as well as on NPC of rat liver, but three to four times more receptors were distributed in PC than NPC. However the amount of TfR on the surface of cell is regulated via synthesis, endocytosis and exocytosis by various factors. Thus it is also important to know that TfRs expressed on the surface membrane of cell are related to the intracellular TfRs.

The TfRs are present in high levels in most rapidly proliferating normal or transformed cells (Girons and Davis, 1989; Keer *et al.*, 1990; akiko *et al.*, 1989). In the regenerating liver, Akiko *et al.* (1989) reported that the numbers of TfRs on the cell surface of hepatocytes were increased at 18 h after partial hepatectomy via increased redistribution to cell membrane and diminished to normal level when the regeneration was over. Thus the TfR is useful surface marker for hepatic proliferation. In this experiment, transferrin binding sites were detected in cell homogenate or cell membrane of NPC as well as PC, and these sites were same types of high affinity sites. Thus we conclude that TfR is distributed in PC and NPC. With the regenerated liver cells after partial hepatectomy, TfRs in cell homogenate and cell membrane of PC or NPC were increased by 3rd day and declined to normal level at 7th day after operation. In spite of some difference with the results of Akiko *et al.* (1989), our results indicate that increased receptor numbers on cell membrane during regeneration are due to not only distribution of intracellular TfRs to the cell surface but also increased intracellular receptor synthesis. And induction of synthesis and distribution of TfRs are terminated as the liver mass is restored.

With the administration of 3-Me-DAB, transferrin binding in homogenate of PC or NPC was increased about 30~80%. Transferrin binding sites in membrane fraction of PC or NPC of 3-Me-DAB treated rat were increased as in that of normal rat liver but not saturated with 15 nM ^{125}I-transferrin, and increased markedly about 3 fold compared to those found in normal rat liver. In contrast to the normal regenerated liver cell homogenate, transferrin binding in the PC or NPC homogenate of 3-Me-DAB treated rat was increased by 7th day after partial hepatectomy. The transferrin binding in PC membrane of 3-Me-DAB treated rat was also further increased by 7th day, whereas those in NPC was increased by 3rd day and reduced to control level at 7th day after partial hepatectomy. The results suggest that carcinogen such as 3-Me-DAB, induces the synthesis of intracellular TfR in the liver and marked redistribution to cell membrame.

In particular, this experiment with 3-Me-DAB treated rat, presented two different classes of TfRs with respect to their different affinities (Kd) for transferrin (Kd: 3.1~4.7 and 25.4~54.1 nM). The one (Kd: 3.1~4.7 nM) fell in with high apparent affinity presented in normal or normal regenerated rat liver cell. These high affinity sites in PC or NPC membrane were increased 30~40% by 3-Me-DAB treatment. The other showed lower affinity (Kd: 25.4~54.1 nM) that was not in normal rat liver or regenerated liver of normal rat.

Sorokin *et al.* (1988), Rothenberger *et al.* (1987) and Ishigura *et al.* (1992) reported that mutant TfR or TfR in transformed cell was changed in function or structure. Although low affinity site of TfR is not yet mentioned by anyone, from the above discussion, we conclude that increased surface TfRs by chemical carcinogen may be due to expressions of abnormal type of receptors (low affnity sites) as well as high affinity sites and marked redistribution to cell membrane.

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=국문초록=

Carcinogen (3-methyl-4-dimethyl-aminoazo benzene) 처리후 간세포막에서의 Transferrin Receptor 변동에 관한 연구

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화학물질에 의한 간암 유발과정에서 transferrin receptor (TfR)의 변동을 밝히기 위해 간을 부분절제한 정상백서의 재생간과 발암물질로 3-Me-DAB를 8주간 투여한 백서 또는 약물 투여 후 부분 간절제 수술을 행하여 세포분열을 유도시킨 백서 간조직으로부터 parenchymal cell (PC)과 nonparenchymal cell (NPC)를 분리하고 각각의 세포막을 제조하여 ^{125}I-transferrin 결합실험을 실시한 바 다음과 같은 성적을 얻었다.

1. 3-Me-DAB 투여에 의하여 간조직에서 oval cell의 증식, 재생성 변화, 결절형성, 담관의 증식 및 담관세포암 등의 현저한 조직학적 변화가 동반되었다. 그러나 간세포증식을 더욱 촉진시키기 위하여 부분간절제 수술을 하였을 때 수술 후 경과에 따른 형태학적 변동은 큰 차이가 없었다.

2. 정상 재생간의 PC 및 NPC homogenate에서 transferrin 결합량은 부분간 절제 수술 후 1일 및 3일에 증가되었으며 수술 후 7일에 정상으로 회복되었다. 3-Me-DAB 투여에 의해 두 세포군에서 모두 정상세포보다 높게 나타났으며 재생기간에 따라 계속 증가되었다.

3. 정상간의 NPC 세포막에서 transferrin 최대 결합량 (Bmax)은 PC 세포막에서 보다 많이 분포되어 있었으며, Kd는 양세포막에서 5.05 또는 6.3 nM로 비슷하였다.

4. 재생간의 NPC 및 PC 세포막에서 transferrin 결합량은 부분 간절제 수술 후 1일 및 3일에 40~50% 증가되었고 수술 후 7일에 정상치로 회복되었다.

5. 3-Me-DAB 처치에 의하여 NPC 및 PC 세포막의 transferrin 결합량은 정상 간세포막에서 보다 약 3배 증가되었고, 3-Me-DAB 투여후 재생간의 NPC 세포막에서는 부분 간절제 수술 후 3일까지 증가된 후 감소되는 양상인데 반해 PC 세포막에서는 수술 후 7일까지 계속 증가되었다.

6. 3-Me-DAB 투여 후 NPC 및 PC 세포막 transferrin binding site에서 Kd치가 3.1~4.7 nM과 25.4~54.1 nM인 두 종류가 존재하는 것으로 나타났다.

이상의 실험성적으로 TfR는 1) 간조직의 PC 및 NPC 세포에 모두 분포되어 있으며, 2) 정상 재생간 및 3-Me-DAB의 처리 후 간세포에서의 세포막 TfR의 증가는 세포내 합성량의 증가에 의하여 일어나며, 3) 정상 재생간의 세포막 TfR는 한 종류의 high affinity site (Kd, <3.1~7.5 nM)에 의하여 증가되나, 3-Me-DAB 처리 후 간세포막에서는 정상에서와 같은 high affinity형 이외에 affinty가 낮은 다른 형태의 TfR (Kd, 25.4~54.1 nM)가 세포막으로 출현됨으로써 크게 증가되는 것으로 사료된다.