Gene Expression of Placental Glutathione S-Transferase in Hereditary Hepatitis and Spontaneous Hepatocarcinogenesis of LEC Strain Rats

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Liver tissues of LEC rats, which develop fulminant hepatitis and hepatocellular carcinoma (hepatoma), were examined by Northern blot analysis using a cDNA probe of rat placental glutathione S-transferase (GST-P). GST-P gene expression was observed not only during hepatocarcinogenesis but also in fulminant hepatitis before development of chronic hepatitis and hepatoma in LEC rats. Cholangiofibrosis in LEC rats also showed high GST-P expression. A transplantable cell line derived from spontaneous LEC hepatoma exhibited a remarkably high expression. By contrast, very weak expression was observed in the livers of young LEC rats before development of hepatitis and control strain rats. Thus, spontaneous hepatic lesions in LEC rats may provide a new clue to elucidate the mechanism of GST-P gene expression.

Key words: LEC rats — Hereditary hepatitis — Spontaneous hepatocarcinogenesis — Placental glutathione S-transferase — Gene expression

The LEC strain rat is a new mutant which develops fulminant hepatitis suddenly about 4 months after birth.^{1,2)} Genetic analysis of LEC rats revealed that the hepatitis has an autosomal recessive mode of inheritance, being ruled by a single gene designated *hts*.³⁾ Although about 30% of rats died of the disease within 1 week after the onset, the remaining 70% survived with chronic hepatitis for more than 1 year and developed well-differentiated hepatocellular carcinomas (hepatomas) together with cholangiofibrosis at a high incidence.⁴⁾ Therefore, it is important to clarify the characteristics of the hepatic lesions in LEC rats. Recently, placental glutathione S-transferase (GST-P) was reported as a new marker enzyme of preneoplastic lesions of rat chemical hepatocarcinogenesis.⁵⁻⁹⁾ Immunohistochemical analysis showed that GST-P-positive foci appeared in the livers of LEC rats at the age of 5 months, that is, after the onset of fulminant hepatitis, and that the number and size of foci increased with age, suggesting that the occurrence of foci may be related to spontaneous development of hepatoma.¹⁰⁾ Therefore, it is worthwhile investigating further the GST-P expression at the molecular level in serial stages of hepatic lesions in LEC rats. In this study, we analyzed gene expression of GST-P in various hepatic lesions of LEC rats using a Northern blot method.

Two inbred strains, LEC and LEA, were isolated from a closed colony of Long-Evans rats maintained at the

Center for Experimental Plants and Animals of Hokkaido University.^{1,2)} LEA rats do not suffer from such liver diseases. Liver tissues were obtained from LEC rats with various hepatic lesions and LEA rats as controls (Table I) and frozen with liquid nitrogen until use. A histopathological description of hepatic lesions in LEC rats was given in our previous reports.^{2,4)} A transplantable LSH206G cell line derived from spontaneous hepatoma of an LEC rat¹¹⁾ was also examined. Total RNAs were extracted from liver tissues of each individual by the guanidinium thiocyanate-CsCl centrifugation method.^{12,13)} Ten μ g of each total RNA was electrophoresed on a 1.1% agarose gel containing 5% formaldehyde^{13,14)} and transferred to an Amersham Hybond N membrane. A cDNA pGP5 probe of rat GST-P¹⁴⁾ was labeled with ³²P-dCTP using an Amersham multi-prime DNA labeling kit. After prehybridization, the membrane was hybridized with the ³²P-labeled probe at 42°C for at least 12 h and washed at 42°C. The membrane was autoradiographed with Fuji New RX film using an intensifying screen at -80°C for 48 h.

Northern blot analysis of total RNAs has revealed a single signal of GST-P of about 0.8 kb with different expression levels among hepatic lesions (Fig. 1). The signals were densitometrically scanned to quantify the relative levels of GST-P expression in different hepatic lesions (Fig. 2). The expression levels of 4- and 12-month-old LEA rats (cases 18 and 19, respectively) were similar to or less than that of 2-month-old (young) LEC rats (cases 1 and 2). The comparison of the average level of each hepatic lesion with that of the young LEC rats

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indicated that the GST-P expression in fulminant hepatitis rats with the same severe jaundice (cases 3–6) was elevated about 4-fold, although there was a slight variation of expression levels among fulminant hepatitis rats.

Table I. Profiles of LEC Rats with Various Hepatic Lesions

Case	Sex ^{a)}	Age (months)	Hepatic lesions ^{b)}
1	F	2	Normal liver
2	F	2	Normal liver
3	M	4	Fulminant hepatitis
4	M	4	Fulminant hepatitis
5	F	4	Fulminant hepatitis
6	M	4	Fulminant hepatitis
7	M	5	Chronic hepatitis
8	F	7	Chronic hepatitis
9	M	8	Chronic hepatitis
10	M	15	T: Hepatoma
11	M	15	T: Hepatoma, N: Nontumorous tissue
12	M	17	T: Hepatoma, N: Nontumorous tissue
13	F	17	T: Hepatoma, N: Nontumorous tissue
14	M	—	Transplantable LSH206G cell line
15	F	17	Cholangiofibrosis
16	M	17	Cholangiofibrosis
17	F	15	Cholangiofibrosis
18	F	4	Normal liver from control LEA rat
19	M	12	Normal liver from control LEA rat

a) Sex: F, female; M, male.

b) Hepatomas in cases 10–13 showed a well-differentiated type without metastasis. A detailed histological description was given in our previous reports.^{2,4)}

Chronic hepatitis rats also showed the GST-P expression at the ages of 5, 7 and 8 months (cases 7–9, respectively). However, the average level in chronic hepatitis decreased to 44% of fulminant hepatitis. Hepatomas in 4 long-surviving LEC rats (cases 10T–13T) showed various expression levels [less than equal (case 13T) to about 13-fold greater (case 10T)], with an average level of about 4-fold, while relatively constant levels of expression were observed in nontumorous liver tissues which included chronic hepatitis and putatively preneoplastic foci (cases

Fig. 2. Relative levels of GST-P gene expression. Histograms indicate average levels of each hepatic lesion relative to an average level of normal LEC rats at 2 months old.

Fig. 1. Northern blot analysis of total RNAs from liver tissues of LEC rats with various hepatic lesions. GST-P bands were present at about 0.8 kb (large arrow), calculated from the positions of 18S (0.2 kb) and 28S (5.1 kb) rRNA. Each lane number corresponds to the case number of Table I.

11N-13N). The transplantable LSH206G cell line (case 14) showed a remarkably high (about 12-fold) expression. Similar variations in the GST-P expression were also reported in chemically induced primary hepatomas and transplantable hepatoma cell lines.⁷⁾ The average expression level in cholangiofibrosis (cases 15-17) was more than 4-fold higher than that in the young LEC rats.

Thus, the present results demonstrate that the GST-P gene was highly expressed not only during hepatocarcinogenesis but also in fulminant hepatitis before development of chronic hepatitis and hepatoma in LEC rats. This new finding of GST-P expression in fulminant hepatitis suggests that the degree of hepatocyte regeneration in hepatitis of LEC rats may be associated with the expression of GST-P gene. In fact, after fulminant hepatitis, the GST-P expression level decreased in chronic hepatitis with mild regeneration of hepatocytes and increased slightly in nontumorous tissues with chronic hepatitis and putatively preneoplastic lesions, although the mechanism of expression of GST-P gene hepatitis remains to be clarified. It has been reported that regenerating livers of partially hepatectomized rats showed weak induction of GST-P.⁷⁾ It is necessary to examine the production of GST-P mRNA in immunohisto-

chemically GST-P-positive as well as negative regions within chronic hepatitis and within nontumorous tissues of the liver with hepatoma. It is also a new finding that cholangiofibrosis, which often developed together with hepatoma in LEC rats, was associated with high expression of GST-P gene. Immunological examinations in previous reports revealed that normal bile ductular cells were weakly GST-P-positive⁹⁾ and that GST-P was detected in chemically induced cholangiocellular lesions of the Syrian hamster.¹⁵⁾ Human GST- π , which is homologous to rat GST-P, was also positively detected in cholangiocarcinomas.⁹⁾

These results indicate that LEC rats associated with spontaneous development of serial hepatic diseases are a promising tool to elucidate the mechanism of GST-P gene expression.

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