Effects of D-Galactosamine Hydrochloride and Partial Hepatectomy on Spontaneous Hepatic Injury and Hepatocarcinogenesis in Long-Evans Cinnamon Rats

Zhongxian Jiao,1 Takamasa Ohnishi,1, 2 Yoshimi Bando,1 Yoshifumi Chone,1 Keisuke Kitaura,1 Hisanori Uehara,1 Yasuo Suzuki,3 Toshikazu Nakamura4 and Keisuke Izumi1, 5

1Second Department of Pathology, 2First Department of Surgery and 3Department of Hygiene, The University of Tokushima School of Medicine, Tokushima 770-8503 and 4Department of Oncology, Biomedical Research Center, Osaka University Medical School, Suita 565-0871

To examine the effect of nongenotoxic chemicals on hepatocarcinogenesis in Long-Evans Cinnamon (LEC) rats, we gave 6-week-old male and female LEC rats (n==18) weekly subcutaneous injections of D-galactosamine hydrochloride (GalN, 300 mg/kg) in 0.9% NaCl or only 0.9% NaCl for 50 weeks, and killed them in week 62. GalN-treated male rats unexpectedly showed no lethal necrotizing hepatitis. GalN treatment increased the incidence of cholangiofibrosis in males and its severity in females, but did not cause significant increases of hepatocellular tumors in either sex. GalN treatment increased the 5-bromo-2′′′′-deoxyuridine (BrdU)-labeling index of hepatocytes and plasma hepatocyte growth factor, and accelerated megalocytic alterations without reduction of the hepatic copper concentration. Next, male and female LEC rats were subjected to two-thirds partial hepatectomy (PH) or sham hepatectomy in week 8 (n==12) or in week 14 (n==9), and killed in week 62. PH in week 14 inhibited lethal hepatitis, but PH in week 8 was less effective. PH reduced the hepatic copper concentration to half that of controls. The present data suggest that induction of hepatocyte regeneration by repeated injections of GalN, or by PH just before the onset of jaundice has a significant effect in prevention of hepatic injury of LEC rats, but not enhancement of spontaneous hepatocarcinogenesis.

Key words: D-Galactosamine hydrochloride — Hepatocyte growth factor — Necrotizing hepatitis — Hepatocarcinogenesis — LEC rat

The LEC rat is an inbred strain showing abnormally high copper accumulation in the liver,11 and has a deletion in the copper-transporting ATPase gene (Atp7b) homologous to the human Wilson’s disease gene.21 About 20–50% of LEC rats die of necrotizing hepatitis with jaundice 4 to 6 months after birth, and the hepatitis is inherited in an autosomally recessive manner.3, 13 Hepatocellular carcinomas develop in rats of 12 months old or more that recover from liver injury.3–5

The oxidative DNA damage by copper ions includes mutagenesis, strand breaks and 8-OHdG formation.6–8 The amounts of 8-OHdG in DNA in the liver and kidney of LEC rats are increased.9 We and other investigators have observed that liver cell injury in LEC rats is increased by phenobarbital,10 clofibrate10 or choline-deficient diet11 and delayed or inhibited by GalN,10 dipyrone,10 L-proline,12 ascorbic acid,12 copper-deficient diet,12, 13 D-penicillamine14 or trientine.15 GalN and dipyrone, an antipyretic drug, are hepatotoxic chemicals, and ascorbic acid is an antioxidant. D-Penicillamine and trientine, copper-chelating agents, inhibit both hepatic injury and liver cancer in LEC rats by reducing the hepatic copper concentration.14–16

GalN has been used for induction of experimental hepatitis in rodents.17, 18 It depletes the uridine nucleotide pool and inhibits RNA synthesis.19 Its administration to rats causes dose-dependent hepatocellular necrosis and compensatory hepatocyte proliferation. PH also induces rapid hepatocyte proliferation.20

In the present study, we investigated 1) the effect of GalN and PH on spontaneous hepatic injury and hepatocarcinogenesis in LEC rats, and 2) the mechanism by which these treatments prevent lethal hepatitis. MATERIALS AND METHODS

Animals LEC/Tj rats were bred in the Institute for Animal Experimentation of the University of Tokushima, in specific pathogen-free conditions. F344/DuCrj rats were obtained from Charles River Japan, Inc., Kanagawa. Animals were housed three to a plastic cage with sterilized
woodchips for bedding in an air-conditioned room at
23±2°C and 55±10% humidity with a 12 h light/dark
cycle, and given pellet diet (Oriental Yeast Co., Tokyo)
and tap water *ad libitum*.

In our laboratory, the mortality rate of untreated LEC
rats during the period of jaundice is 12.4% for males
(*n*=193) and 42.3% for females (*n*=52).

**Experiment 1** Six-week-old male and female LEC
rats (*n*=18) were given weekly subcutaneous injections of 300
mg/kg of GalN (Sigma Chemical Co., St. Louis, MO) dis-
solved in 0.9% NaCl, or 0.9% NaCl only (2 ml/kg body
weight) for 50 weeks. The dose of GalN was chosen from
the doses which we have used in previous carcinogenicity
studies using F344 rats (unpublished data). Animals were
examined daily for the emergence of jaundice and their
body weight was recorded once a week. All surviving rats
were killed under ether anesthesia in week 62 and all
organs including all hepatic lobes were fixed in 10% buff-
nered formalin and embedded in paraffin. Sections were
stained with hematoxylin and eosin, and examined histo-
logically. Histological types of liver tumors were classi-
fied as reported.21)

**Experiment 2** Six-week-old male LEC rats were given
weekly subcutaneous injections of GalN (300 mg/kg) in
0.9% NaCl or 0.9% NaCl only for 5, 12 or 25 weeks (*n*=5
at each point). They were killed 7 days after the final
injection, and the copper concentration of their liver was
measured.

**Experiment 3** The labeling index with BrdU (Sigma
Chemical Co.) of hepatocytes after GalN treatment was
measured. Six-week-old male LEC rats (*n*=5 at each
point) were given weekly subcutaneous injections of GalN
(750 mg/kg or 300 mg/kg) in 0.9% NaCl or 0.9% NaCl only for three weeks. On days 0, 2, 3 and 7 after the final
injection, rats were given an intraperitoneal injection of
50 mg/kg of BrdU and killed 30 min later. The liver was
fixed in 95% ethanol and embedded in paraffin. Sections
were stained with anti-BrdU antibody (Becton Dickinson
Immunocytochemistry Systems, Mountain View, CA) using a
DAKO LSAB kit, peroxidase (DAKO Co., Carpinteria,
CA) after partial denaturation of double-stranded DNA.
Livers were also fixed in buffered formalin and examined
histologically.

**Experiment 4** Six-week-old male LEC rats were given
weekly subcutaneous injections of GalN (300 mg/kg) in
0.9% NaCl or 0.9% NaCl only for three weeks (*n*=4 at
each point). Rats were killed on day 0, 1, 2 or 3 after the
final injection, and their plasma HGF concentration was
measured by sandwich enzyme-linked immunosorbent
assay (rat HGF-EIA kit, Institute of Immunology, Tokyo).
Frozen sections of the liver were fixed in ice-cold acetone
for 10 min and stained with polyclonal anti-rat HGF anti-
body using a DAKO LSAB kit.

**Experiment 5** Male and female LEC rats were subjected
to PH20) or SH at 8 weeks (*n*=12) or 14 weeks (*n*=9). Rats
were killed in week 62 and examined histologically.

**Experiment 6** Ten-week-old male LEC and F344 rats
were subjected to PH or SH and killed on day 0, 3, 7 or
14 (*n*=3 at each point). The liver/body weight and hepatic
copper concentration were measured. The liver was exam-
ined histologically.

**Analysis of copper concentration** Samples of the liver
(0.5 g) were stored at −80°C until use. They were ashed
with nitric acid, and their copper concentrations were
measured in an atomic absorption spectrophotometer
(AA-782, Nippon Jarrel Ash, Co., Kyoto).

**Statistical analyses** Data on relative liver weights, the
numbers of tumors and BrdU labeling indices were ana-

---

Fig. 1. Survival rates (A) and growth curves (B) of male and
female LEC rats given weekly subcutaneous injections of 300
mg/kg of GalN in 0.9% NaCl or only 0.9% NaCl for 50 weeks
(Experiment 1). Surviving rats were killed in week 62 after a 6-
week recovery period. — GalN, males; — 0.9% NaCl, males;
— GalN, females; — 0.9% NaCl, females; □ inhibition of
body weight gain.
RESULTS

Long-term test on GalN  In Experiment 1, the incidence of jaundice in GalN-treated rats was higher than that in control rats. None of the GalN-treated male rats died, but three control male rats died of necrotizing hepatitis between week 19 and 31, five GalN-treated female rats between week 14 and 30, and 12 control female rats between week 16 and 21 (Fig. 1A). One 0.9% NaCl-treated male rat died of cecal ulcer in week 43, and one GalN-treated female rat died of hepatocellular carcinoma with lung metastasis in week 60. The survival rate of males was higher than that of females, and the rates in GalN-treated rats of both sexes were higher than those of the controls. Inhibition of body weight gain of rats with jaundice was seen twice in control female rats and once in control males and GalN-treated females, but not in GalN-treated males (Fig. 1B). The average number of gross hepatocellular tumors (≥5 mm in diameter) in GalN-treated males was higher than that in control males, the difference not being significant (Table I). Hepatocellular carcinoma developed at low incidence in only GalN-treated rats. The liver/body weight ratio was higher in GalN-treated animals of both sexes because of cholangiofibrosis, and its incidence in GalN-treated males was higher than that in control males (P<0.01).

| Table I. Effect of GalN on Carcinogenesis of LEC Rats (Experiment 1) |
|---|---|---|---|---|---|---|---|---|
| Sex | Treatment | Initial no. of rats | Jaundice | Effective no. of rats | Liver/body weight (%) | No. of tumors/rat (≥5 mm) | Hepatocellular adenoma | Hepatocellular carcinoma |
|---|---|---|---|---|---|---|---|---|
| M | GalN | 18 | 3 (17%) | 18 | 5.6±3.2 | 1.4±1.8 | 12 (67%) | 1 (6%) |
| | 0.9% NaCl | 18 | 13 (72%) | 14 | 3.3±0.7 | 0.5±0.6 | 7 (50%) | 0 |
| F | GalN | 18 | 17 (94%) | 13 | 9.5±3.6 | 0.5±0.8 | 4 (31%) | 1 (8%) |
| | 0.9% NaCl | 18 | 18 (100%) | 6 | 8.1±2.2 | 0.5±0.5 | 3 (50%) | 0 |

a) ≥60 weeks old.
b, c) Significantly different from the 0.9% NaCl group at \( P<0.01 \) and \( P<0.001 \) (Fisher’s exact probability test).
d) Mean±SD.
e) Significantly different from the 0.9% NaCl group at \( P<0.02 \) (Student’s t test).
f) One renal cell carcinoma and one thymic carcinoma.
Hepatic copper concentrations of GalN-treated rats In Experiment 2, the average copper concentrations in the liver at all times were similar in GalN-treated and control rats (Fig. 2). Serum aspartate aminotransferase and alanine aminotransferase measured with an automatic analyzer were similar in the two groups (data not shown).

Effect of GalN on cell proliferation In Experiment 3, the BrdU labeling index of hepatocytes in rats treated with 300 mg/kg of GalN was 4.6 times that in the controls on day 2 (P<0.01), and decreased to close to the control level on day 7 (Fig. 3). Histologically, liver cell enlargement with large nuclei, which is always observed in LEC rats recovering from jaundice, was observed in GalN-treated rats, especially in the 750 mg/kg-treated group, on day 3 (Fig. 4).

Plasma HGF concentration and immunohistochemistry of the liver in GalN-treated rats In Experiment 4, the plasma HGF increased on days 1 and 2 and decreased to near the normal level on day 2 except in one rat (Fig. 5).
Immunohistochemically, slight increases of the number of HGF-positive mesenchymal cells were observed in GalN-treated rats on day 2.

**Effect of PH on spontaneous liver injury and hepatocarcinogenesis** In Experiment 5, PH completely prevented death in the period of jaundice in males at both 8 and 14 weeks (Fig. 6). PH increased the survival rate of females of 14 weeks, but not 8 weeks. PH decreased the liver/body weight ratio by preventing severe cholangiofibrosis in females of both 8 and 14 weeks, although the incidences of cholangiofibrosis were similar in these groups (Table II). Hepatocellular and cholangiocellular carcinomas developed at low incidences in only PH groups of both sexes.

**Hepatic copper concentrations of heptatectomized rats** In Experiment 6, the liver/body weight ratios in heptatectomized F344 rats recovered to 97% of that of sham-operated F344 rats on day 14, but that of LEC rats was 86%
Fig. 7. Liver/body weight (A) and hepatic copper concentration (B) of 10-week-old male LEC and F344 rats \( n = 3 \) after PH or SH (Experiment 6). Rats were killed on days 0, 3, 7 and 14. • LEC, PH; ○ LEC, SH; ■ F344, PH; □ F344, SH.

Fig. 8. Histological appearances on day 3 of the liver of hepatectomized and sham-operated rats (Experiment 6). (A) LEC rat, PH, (B) LEC rat, SH, (C) F344 rat, PH, (D) F344 rat, SH (H & E, ×150).
of the control level (Fig. 7). In LEC rats, the copper concentration of hepatectomized rats was 45% of that of control rats on day 14. Histologically, nuclear enlargement in hepatectomized LEC rats was more prominent than that in hepatectomized F344 rats (Fig. 8).

**DISCUSSION**

Excess copper accumulation in hepatocytes induces necrotizing hepatitis with jaundice in LEC rats. Necrotizing hepatitis is reported to be more severe in females than in males, as also shown in the present study. Although the severity of hepatic injury is related to the hepatic copper concentration, it is unknown why lethal hepatitis develops 4 to 6 months after birth. In the present study, we demonstrated that 1) both long-term administration of GalN and PH in LEC rats of 8 or 14 weeks old inhibited the emergence of jaundice and increased the survival rates, 2) both GalN and PH induced hepatocyte proliferation, 3) PH reduced the hepatic copper concentration for at least two weeks, but GalN did not reduce it. These results suggest that hepatic regeneration induced by GalN or PH plays a more significant role than decrease of the hepatic copper concentration in preventing severe hepatic injury. In a recent study, we observed that short-term administration of a necrogenic dose of N-diethylnitrosamine, a potent hepatocarcinogen, also reduced the mortality of LEC rats (unpublished data).

HGF is a potent mitogen and a cytoprotective agent for hepatocytes. There are reports that GalN increases HGF mRNA in the liver of rats, and that HGF works as an anti-hepatitis agent against GalN-induced liver injury of HGF transgenic mice. In the present study, GalN treatment increased the plasma HGF on day 1. There are also reports that in LEC rats 1) plasma HGF, HGF mRNA expression and HGF-positive non-parenchymal cells in the liver increase during the phase of fulminant hepatitis, and decrease during the chronic phase, and 2) HGF-positive cells increase in the liver 24 h after PH. These findings also support the idea that HGF production by GalN treatment may be related to the low mortality rate of LEC rats in the present study.

Characteristic histological findings on hepatocytes of LEC rats after the period of jaundice were megalocytic alteration and cholangiofibrosis, the latter being more severe in females than in males. Megalocytic hepatocytes are polyploid cells induced by intracytoplasmic copper accumulation, and the DNA content of these cells reaches 64n. Proliferation of megalocytic hepatocytes is low, and most of these cells were not labeled on administration of BrdU for one week with a mini-osmotic pump (unpublished data). GalN treatment induced earlier development of megalocytic alteration of hepatocytes without reduction of hepatic copper accumulation than 0.9% NaCl treatment in the present study. Thus, megalocytic alteration may occur as a protective reaction against the toxicity of GalN as well as the process of hepatocyte regeneration.

Cholangiofibrosis is induced by various hepatocarcinogens and hepatotoxic agents including GalN. Long-term administration of copper-chelating agents reduces copper and 8-OHdG in the liver, and inhibits cholangiofibrosis in LEC rats. In the present study, cholangiofibrosis was enhanced by GalN without reduction in hepatic copper accumulation, and inhibited by PH, which reduced hepatic copper accumulation. The hydroxy radical and/or another fibrogenic factor(s) may be related to the development of cholangiofibrosis.

Cell proliferation is an important factor in the carcinogenicities of nongenotoxic compounds, but toxic agents are not necessarily carcinogenic in two-year carcinogenicity bioassays. Recently, using male F344 rats, we found that hepatocellular carcinomas were not induced by repeated injections of GalN alone for more than 70 weeks, but that after initiation with N-diethylnitrosamine, GalN enhanced hepatocarcinogenesis (unpublished data). At first, we supposed that GalN would enhance hepatocarcinogenesis in LEC rats, because liver tumors develop spontaneously in this strain of rats, and it is suspected that LEC rats have a cancer susceptible gene(s). However, in the present study we could not show that repeated injections of GalN without an initiator enhanced hepatocarcinogenesis. Therefore, in these rats predisposing conditions, such as a cancer susceptibility gene(s) and copper accumulation, may be insufficient for GalN-induced tumor promotion, or a longer observation period may be necessary to induce liver cancer because in this study hepatocellular carcinomas developed at low incidence in GalN-treated and hepatectomized rats, but not in the respective controls.

This study showed that GalN and PH induced early development of megalocytic alteration, and GalN enhanced cholangiofibrosis in LEC rats. Both GalN and PH reduced the severity of necrotizing hepatitis, but did not enhance hepatocarcinogenesis under the present experimental conditions. These findings suggest that hepatocyte regeneration and/or reduction of hepatic copper accumulation are significant in preventing lethal hepatitis in LEC rats.

(Received January 25, 1999/Accepted March 8, 1999)
REFERENCES

1) Li, Y., Togashi, Y., Sato, S., Emoto, T., Kang, J.-H., Takeichi, N., Kobayashi, H., Kojima, Y., Une, Y. and Uchino, J. Spontaneous hepatic copper accumulation in Long-Evans Cinnamon rats with hereditary hepatitis. J. Clin. Invest., 87, 1858–1861 (1991).

2) Wu, J., Forbes, J. R., Chen, H. S. and Cox, D. W. The LEC rat has a deletion in the copper transporting ATPase gene homologous to the Wilson disease gene. Nat. Genet., 7, 541–545 (1994).

3) Yoshida, M. C., Masuda, R., Sasaki, S., Takeichi, N., Kobayashi, H., Dempo, K. and Mori, M. New mutation causing hereditary hepatitis in the laboratory rat. J. Hered., 78, 361–365 (1987).

4) Masuda, R., Yoshida, M. C., Sasaki, M., Dempo, K. and Mori, M. High susceptibility to hepatocellular carcinoma development in LEC rats with hereditary hepatitis. Jpn. J. Cancer Res., 79, 828–835 (1988).

5) Sawaki, M., Enomoto, K., Takahashi, H., Nakajima, Y. and Mori, M. Phenotype of preneoplastic and neoplastic liver lesions during spontaneous liver carcinogenesis of LEC rats. Carcinogenesis, 11, 1857–1861 (1990).

6) Sagripanti, J. L. and Kraemer, K. H. Site-specific oxidative DNA damage at polyguanosines produced by copper plus hydrogen peroxide. J. Biol. Chem., 264, 1729–1734 (1989).

7) Tkeshelashvili, L. K., McBride, T., Spence, K. and Loeb, L. A. Mutation spectrum of copper-induced DNA damage. J. Biol. Chem., 266, 6401–6406 (1991).

8) Toyokuni, S. and Sagripanti, J. L. Association between 8-hydroxy-2′-deoxyguanosine formation and DNA strand breaks mediated by copper and iron. Free Radic. Biol. Med., 20, 859–864 (1996).

9) Yamamoto, F., Kasai, H., Togashi, Y., Takeichi, N., Hori, T. and Nishimura, S. Elevated level of 8-hydroxydeoxyguanosine in DNA of liver, kidneys, and brain of Long-Evans Cinnamon rats. Jpn. J. Cancer Res., 84, 508–511 (1993).

10) Izumi, K., Uehara, H., Otsuka, H. and Matsumoto, K. Inhibitory and intensifying effects of long-term exposure to chemicals on spontaneous hepatic injury in LEC rats. In “The LEC Rat: A New Model for Hepatitidis and Liver Cancer,” ed. M. Mori, M. C. Yoshida, N. Takeichi and N. Taniguchi, pp. 114–119 (1991). Springer-Verlag, Tokyo.

11) Sugiyama, T., Matsunaga, M., Jain, S. K., Jain, S., Ikeda, Y. and Taniguchi, N. Enhancing effect of a choline-deficient diet on alterations of hepatic drug-metabolizing enzymes in hepatitis- and hepatoma-predisposed rats (LEC rats). Jpn. J. Cancer Res., 82, 390–396 (1991).

12) Hawkins, R. L., Mori, M., Inoue, M. and Torii, K. Proline, ascorbic acid, or thioredoxin affect jaundice and mortality in Long Evans Cinnamon rats. Pharmacol. Biochem. Behav., 52, 509–515 (1995).

13) Sawaki, M., Hattori, A., Tsuzuki, N., Sugawara, N., Enomoto, K., Sawada, N. and Mori, M. Chronic liver injury promotes hepatocarcinogenesis of the LEC rat. Carcinogenesis, 19, 331–335 (1998).

14) Togashi, Y., Li, Y., Kang, J.-H., Takeichi, N., Fujioka, Y., Nagashima, K. and Kobayashi, H. D-Penicillamine prevents the development of hepatitidis in Long-Evans Cinnamon rats with abnormal copper metabolism. Hepatology, 15, 82–87 (1992).

15) Sone, H., Maeda, M., Wakabayashi, K., Takeichi, N., Mori, M., Sugimura, T. and Nagao, M. Inhibition of hereditary hepatitis and liver tumor development in Long-Evans Cinnamon rats by the copper-chelating agent trientine dihydrochloride. Hepatology, 23, 764–770 (1996).

16) Kang, J.-H., Togashi, Y., Kasai, H., Hosokawa, M. and Takeichi, N. Prevention of spontaneous hepatocellular carcinoma in Long-Evans Cinnamon rats with hereditary hepatitis by the administration of D-penicillamine. Hepatology, 18, 614–620 (1993).

17) Keppler, D., Lesch, R., Reutter, W. and Decker, K. Experimental hepatitis induced by D-galactosamine. Exp. Mol. Pathol., 9, 279–290 (1968).

18) Decker, K. and Keppler, D. Galactosamine induced liver injury. In “Progress in Liver Diseases IV.” ed. H. Popper and F. Schaffner, pp. 183–199 (1972). Grune and Stratton, New York.

19) Keppler, D. O. R., Pausch, J. and Decker, K. Selective uridine triphosphate deficiency induced by D-galactosamine in liver and reversed by pyrimidine nucleotide precursors. J. Biol. Chem., 249, 211–216 (1974).

20) Higgins, G. M. and Anderson, R. M. Experimental pathology of the liver—I. Restoration of the liver of the white rat following partial surgical removal. Arch. Pathol., 12, 186–202 (1931).

21) Bannasch, P. and Zerban, H. Tumors of the liver. In “Pathology of Tumors in Laboratory Animals, 2nd Ed., Vol. 1—Tumors of the Rat.” ed. V. Turusov and U. Mohr, pp. 199–240 (1990). IARC, Lyon.

22) Kasai, N., Kamimura, E., Miyoshi, I. and Yoshida, M. C. Reproductive performance and effects of pregnancy on the acute phase of hepatitis in LEC rats. In “The LEC Rat: A New Model for Hepatitis and Liver Cancer,” ed. M. Mori, M. C. Yoshida, N. Takeichi and N. Taniguchi, pp. 11–19 (1991). Springer-Verlag, Tokyo.

23) Kinoshita, T., Tashiro, K. and Nakamura, T. Marked increase of HGF mRNA in non-parenchymal liver cells of rats treated with hepatotoxins. Biochem. Biophys. Res. Commun., 165, 1229–1234 (1989).

24) Okano, J., Shiota, G. and Kawasaki, H. Protective action of hepatocyte growth factor for acute liver injury caused by D-galactosamine in transgenic mice. Hepatology, 26, 1241–1249 (1997).

25) Nakayama, N., Kashiwazaki, H., Kobayashi, N., Hamada, J., Ogiso, Y., Itakura, Y., Matsumoto, K., Nakamura, T., Koike, T., Kuzumaki, N. and Takeichi, N. Hepatocyte growth factor and c-met expression in Long-Evans Cinna-
mon rats with spontaneous hepatitis and hepatoma. *Hepatology*, **24**, 596–602 (1996).

26) Fujimoto, Y., Oyamada, M., Hattori, A., Takahashi, H., Sawaki, M., Dempo, K., Mori, M. and Nagao, M. Accumulation of abnormally high ploid nuclei in the liver of LEC rats developing spontaneous hepatitis. *Jpn. J. Cancer Res.*, **80**, 45–50 (1989).

27) Lesch, R., Bauer, C. and Reutter, W. The development of cholangiofibrosis and hepatomas in galactosamine induced cirrhotic rat liver. *Virchows Arch. B Cell Pathol.*, **12**, 285–289 (1973).

28) Tatematsu, M., Kaku, T., Medline, A. and Farber, E. Intestinal metaplasia as a common option of oval cells in relation to cholangiofibrosis in liver of rats exposed to 2-acetylaminofluorene. *Lab. Invest.*, **52**, 354–362 (1985).

29) Elmore, L. W. and Sirica, A. E. Phenotypic characterization of metaplastic intestinal glands and ductular hepatocytes in cholangiofibrotic lesions rapidly induced in the caudate liver lobe of rats treated with furan. *Cancer Res.*, **51**, 5752–5759 (1991).

30) Cohen, S. M. and Ellwein, L. B. Cell proliferation in carcinogenesis. *Science*, **249**, 1007–1011 (1990).

31) Tennant, R. B., Elwell, M. R., Spalding, J. W. and Griesemer, R. A. Evidence that toxic injury is not always associated with induction of chemical carcinogenesis. *Mol. Carcinog.*, **4**, 420–440 (1991).

32) Ward, J. M., Uno, H., Kurata, Y., Weghorst, C. M. and Jang, J.-J. Cell proliferation not associated with carcinogenesis in rodents and humans. *Environ. Health Perspect.*, **101** (Suppl. 5), 125–136 (1993).

33) Hattori, A., Sawaki, M., Enomoto, K., Tsuzuki, N., Isomura, H., Kojima, T., Kamibayashi, Y., Sugawara, N., Sugiyama, T. and Mori, M. The high hepatocarcinogen susceptibility of LEC rats is genetically independent of abnormal copper accumulation in the liver. *Carcinogenesis*, **16**, 491–494 (1995).