Role of Copper Accumulation in Spontaneous Renal Carcinogenesis in Long-Evans Cinnamon Rats

Keisuke Kitaura,1 Yoshifumi Chone,1 Nobuo Satake,1 Akiko Akagi,1 Takamasa Ohnishi,1 Yasuo Suzuki2 and Keisuke Izumi1, 3

1Second Department of Pathology and 2Hygiene, The University of Tokushima School of Medicine, 3-18-15 Karamoto-cho, Tokushima 770-8503

Spontaneous renal cell tumors in totals of 223 male and female Long-Evans Cinnamon (LEC) rats of 51–120 weeks old, 157 male F344 rats of 51–120 weeks old, and 14 male Long-Evans Agouti (LEA) rats of 51–70 weeks old were examined histologically. The incidences of renal cell tumors increased with age in male and female LEC rats, but no tumors developed in F344 or LEA rats. Dilated atypical tubules of the kidneys were observed at high incidence in aged LEC rats. Copper staining of LEC rat kidneys showed a positive reaction in proximal tubules of the cortex and the outer stripe of the medulla. The renal copper concentration of LEC rats reached a peak in the period of necrotizing hepatitis with renal tubular necrosis, and was higher than that in F344 rats for up to 106 weeks. In contrast, the renal iron concentration of LEC rats was lower than that in F344 rats except in the period of necrotizing hepatitis. Long-term treatment of LEC rats with D-penicillamine, a copper-chelating agent, inhibited accumulation of copper, but not iron, in the kidneys, and inhibited the development of karyomegaly of proximal tubules and dilated atypical tubules. These results suggest that persistent copper accumulation after toxic necrosis of tubules is the major cause of spontaneous renal carcinogenesis in LEC rats.

Key words: Renal carcinogenesis — Spontaneous — Copper — LEC rat

The LEC rat is an inbred strain showing abnormally high copper accumulation in the liver, and has a deletion in the copper-transporting ATPase gene (Atp7b) homologous to the human Wilson’s disease gene. This mutant rat strain was originally isolated from a closed colony of Long-Evans rats. LEC rats develop necrotizing hepatitis with jaundice 4 to 6 months after birth and this hepatitis is inherited in an autosomally recessive manner. About 20–50% of these rats die of fulminant hepatitis, and hepatocellular carcinomas develop in rats of 12 months old or more that recover from the liver injury. Both the hepatitis and hepatocellular carcinomas can be prevented by treatment with the copper-chelating agent, D-penicillamine or trientine.

Patients with Wilson’s disease are known to have abnormal renal function and penicillamine therapy improves their renal function, but we know of no reports of renal neoplasms in cases of Wilson’s disease. We have reported that renal cell tumors develop spontaneously in LEC rats. In the present study, we examined preneoplastic and neoplastic lesions of the kidneys in rats of 51–120 weeks old, and the changes in renal copper and iron concentrations of LEC and F344 rats of 6–106 weeks old. We also investigated the effect of D-penicillamine on spontaneous renal carcinogenesis.

MATERIALS AND METHODS

Animals LEC/Tj rats and LEA/Tj rats, a sibling line of the LEC rats, were bred in the Institute for Animal Experimentation of the University of Tokushima, in specific pathogen-free conditions. In our laboratory, the mortality rate of LEC rats during the period of jaundice is 12.4% for males (n=193) and 42.3% for females (n=52). 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.

Spontaneous renal cell tumors For examining spontaneous renal cell tumors, we used totals of 186 male and 37 female LEC rats of 51–120 weeks old, 157 male F344 rats of 51–120 weeks old, and 14 male LEA rats of 51–70 weeks old. All rats were untreated controls used for carcinogenicity studies or kept for spontaneous tumorigenesis studies in our laboratory over the last 6 years. Therefore, the number of aged LEC rats was limited. Animals were killed by cervical arteriotomy under ether anesthesia and blood was removed by aortotomy. One horizontal slice of

---

*To whom correspondence should be addressed.
E-mail: izumi@basic.med.tokushima-u.ac.jp
Abbreviations: LEC, Long-Evans Cinnamon; LEA, Long-Evans Agouti; BrdU, 5-bromo-2′-deoxyuridine; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.
each kidney, and other major organs were fixed in 10% buffered formalin (pH 7.4), embedded in paraffin, sectioned and stained with hematoxylin and eosin for histological examination. In addition, the liver and kidneys of selected animals were stained for copper deposition by a modification of Timm’s method. Histological types of renal cell tumors were classified as reported. 

Statistical analyses Data on copper and iron concentrations in the kidney were analyzed by using Student’s t test and the incidences of preneoplastic and neoplastic lesions were analyzed by means of Fisher’s exact probability test.

RESULTS

Spontaneous liver tumors The incidences of liver tumors in LEC, F344 and LEA rats are shown in Table I. Hepatocellular tumors (hepatocellular adenomas plus carcinomas) developed in most LEC rats of over 71 weeks. Hepatocellular tumors were very rare in F344 rats until 120 weeks old, and were absent in LEA rats of up to 70 weeks old.

Spontaneous kidney tumors and dilated atypical tubules Renal cell adenomas were microscopic in size. Fig. 1A shows an intracystic renal cell adenoma. Renal cell carcinomas were larger than adenomas and characterized by areas of necrosis, sarcomatous change and invasive growth (Fig. 1B). All gross renal cell carcinomas developed in male LEC rats, and their diameters were 35 mm (63 weeks), 6 mm (65 weeks), 13 mm (74 weeks), 15 mm (79 weeks), 15 mm (82 weeks), 10 mm (99 weeks), 2 and 4 mm (105 weeks), 8 mm (111 weeks), 15 mm (112 weeks), and 30 mm (113 weeks). No mesenchymal or embryonal tumors were observed in this study. The incidence of renal cell tumors (renal cell adenomas plus carcinomas) increased with age in both sexes, those in male and female LEC rats being 8/147 (5%) in weeks 51–70, 5/37 (14%) in weeks 71–90, 4/24 (17%) in weeks 91–110, and 8/15 (53%) in weeks 111–120. No tumors developed in F344 or LEA rats during the observation periods (Table II).

Massive renal tubular necrosis occurred during the period of jaundice (Fig. 1C). Karyomegaly of the proximal tubules was observed in all LEC rats examined (Fig. 2A). Copper staining of the kidneys of LEC rats demonstrated copper deposition in the proximal tubules in the

| Strain | Sex | No. of rats | Age (weeks) |
|--------|-----|-------------|-------------|
|        |     | 51–60 | 61–70 | 71–80 | 81–90 | 91–100 | 101–110 | 111–120 |
| LEC    | M   | 186  | 7/46 (15%) | 32/71 (45%) | 21/27 (78%) | 6/7 (86%) | 10/10 (100%) | 12/12 (100%) | 13/13 (100%) |
|        | F   | 37   | —        | 13/30 (43%) | 2/2 (100%) | 1/1 (100%) | 1/2 (50%) | —        | 2/2 (100%) |
| F344   | M   | 157  | 0/42 | 0/9 | 0/4 | 1/38 (3%) | 0/24 | 0/4 | 0/36 |
| LEA    | M   | 14   | 0/11 | 0/3 | — | — | — | — | — |

a) Hepatocellular adenomas plus carcinomas.
b) Two rats with hepatocellular carcinomas had metastases to the lung and adrenal gland, respectively.
c–e) Significantly different from F344 rats by Fisher’s exact probability test: c) P<0.001, d) P<0.01, e) P<0.02.
cortex and the outer stripe of the medulla (Fig. 3). Dilated atypical tubules lined by single or multiple layers of acidophilic cells (Fig. 1D, 1E) were found in more than half the LEC rats of more than 81 weeks old, but only in one F344 rat (Table III). Some of the proliferating lining cells of the dilated atypical tubules stained positively...
with BrdU (Fig. 1F). So-called nephropathy, which is often observed in aged F344 rats, was absent or mild in LEC rats.

Copper and iron concentrations in the kidneys

The copper concentration in the kidneys of LEC rats increased dramatically in week 20 when necrotizing hepatitis occurred to 170±30 µg/g wet weight, which was 24 times that in F344 rats (7±1 µg/g, Fig. 4). Its concentration decreased markedly in week 36 (26±3 µg/g), but was still significantly higher than that in F344 rats in weeks 64 and 106. The iron concentration in the kidneys of LEC rats also reached a peak in week 20 (140±41 µg/g) and then decreased gradually. On the other hand, the iron concentration in F344 rats increased with age. After about 1 year, the iron concentration in F344 rats became higher than that in LEC rats.

**Effect of D-penicillamine**

Two rats which died of unknown causes in week 55 (untreated) and in week 45 (D-penicillamine-treated) were excluded from the effective numbers (Table IV). Karyomegaly of proximal tubules, dilated atypical tubules and renal cell adenoma developed in untreated LEC rats, whereas none of these renal lesions developed in D-penicillamine-treated rats (Fig. 2). The incidence of liver tumors was also decreased in D-penicillamine-treated rats. On D-penicillamine treatment, the copper concentration in the liver was decreased at 56 weeks, but the iron concentration was increased significantly (Fig. 5). Copper concentration in the kidneys was

---

Table II. Incidences of Spontaneous Renal Cell Tumors in LEC, F344 and LEA Rats

| Strain | Sex | No. of rats | Age (weeks) | Histology          | Incidences |
|--------|-----|-------------|-------------|-------------------|------------|
|        |     |             | 51–60       | 61–70             |            |
| LEC    | M   | 186         | 2/46 (4%)   | 3/71 (4%)         | 3/27 (11%) |
|        |     |             | 2 (4%)      | 1 (1%)            | 1 (4%)     |
|        |     |             |             |                   | 0          |
|        |     |             |             |                   | 1 (10%)    |
|        |     |             |             |                   | 1 (8%)     |
|        |     |             |             |                   | 4 (31%)    |
| F      | 37  | renal cell tumor | — | 3/30 (10%) | 1/2 (50%) |
|        |     | adenoma      | — | 3 (10%)    | 0          |
|        |     | carcinoma    | — | 0          | 1 (50%)   |
|        |     |             |             |                   | 0          |
| F344   | M   | 157         | 0/42        | 0/9               | 0/4        |
|        |     | renal cell tumor | — | —         | —          |
| LEA    | M   | 14          | 0/11        | 0/3               | —          |

* Renal cell adenoma plus renal cell carcinoma.
* Two rats had both adenoma and carcinoma.
* c, d) Significantly different from F344 rats by Fisher’s exact probability test: c) P<0.001, d) P<0.01.

---

Fig. 2. Proximal tubules of 62-week-old untreated and D-penicillamine-treated LEC rats (H & E, ×198). (A) Karyomegaly in an untreated rat, (B) no apparent karyomegaly in a D-penicillamine-treated rat.
reduced by D-penicillamine treatment ($P<0.001$), but the iron concentration was not.

**DISCUSSION**

Spontaneous renal cell tumors are uncommon in rats. The incidence of tumors (renal cell adenomas and carcinomas) is higher in males than in females. Their incidences in 2-year carcinogenicity studies were reported to be $0.3–0.6\%$ in male and $0.1–0.3\%$ in female F344 rats, and $0.1–1.2\%$ in male and $0\%$ in female Sprague-Dawley rats. In the present study, no male F344 rats developed renal cell tumors, but both sexes of LEC rats developed renal cell tumors at high incidence, with no sex difference in the incidence. The Eker rat, which has a germline mutation in the rat homologue ($Tsc2$) of the human tuberous sclerosis gene, is a hereditary cancer model and develops multicentric and bilateral renal cell tumors spontaneously at high incidence by 1 year of age. The incidence and multiplicity of
renal cell tumors in LEC rats are lower than those in Eker rats, and tumors in LEC rats are likely to develop later in life.

Copper and iron are potent generators of reactive oxygen species in vivo, and these reactive oxygen species induce DNA damage. Both metallothionein-bound and unbound copper increased in the liver and kidneys of LEC rats with jaundice, and the amount of the latter, which plays a crucial role in copper toxicity, is well correlated with the total cytosolic copper content in these organs.

It has been suggested that hepatic metallothionein-bound copper in LEC rats generates hydroxy-radicals in the presence of hydrogen peroxide in the cells. We measured the total copper and iron concentrations in these organs.

The oxidative DNA damage by copper ions includes mutagenesis, strand breaks and 8-OHdG formation. The amount of 8-OHdG in DNA is increased in the liver and kidney of LEC rats, and the levels of Cu,Zn-superoxide dismutase, a specific scavenger of superoxide, in LEC rats are lower than those in Wistar rats, especially in the liver and kidney. Copper and/or hemolysis-induced iron accumulation are considered to play roles in hepatocarcinogenesis in LEC rats, because copper-induced hemolysis occurs in the period of necrotizing hepatitis, and iron-deficient diet prevents the development of liver tumors. In the present study, despite the diffuse periportal hemosiderosis of hepatocytes (data not shown) and the increased hepatic iron concentration the incidence of hepatocellular tumors in d-penicillamine-treated LEC rats was lower than that in controls, although we did not measure metal concentrations during the period of jaundice.

Recently, repeated i.p. injections of copper (cupper nitrolotriacetate) were reported to induce renal cell carcinomas in Wistar rats. Hemosiderosis caused by copper-induced hemolysis was also suggested to play a role in copper-induced renal carcinogenesis. In the present study, we observed that a significantly higher renal copper concentration and lower iron concentration in LEC rats than in F344 rats continued until at least 2 years old, and long-term d-penicillamine administration inhibited the development of proximal tubule karyomegaly, dilated atypical tubules, and copper, but not iron accumulation in the kidneys. In untreated F344 rats, no renal tumors developed and dilated atypical tubules only rarely developed despite

| Treatment     | No. of rats | Body weight | Hepatocellular tumor* | Karyomegaly | Dilated atypical tubules | Renal cell tumor |
|---------------|-------------|-------------|-----------------------|-------------|--------------------------|-----------------|
| none          | 13          | 346±30b     | 5 (38%)               | 13 (100%)   | 5 (38%)                  | 1 (8%)          |
| d-penicillaminec) | 13          | 361±21      | 1 (8%)                | 0           | 0                        | 0               |

*a) Hepatocellular adenoma plus carcinoma.
*b) Mean±SD.
*c) Six-week-old male LEC rats were treated with 0.07% d-penicillamine 5 days a week for 56 weeks.
*d, e) Significantly different from untreated controls by Fisher’s exact probability test: d) P<0.001, e) P<0.05.

Fig. 5. Copper and iron concentrations in week 56 in the liver and kidneys of LEC rats with and without d-penicillamine treatment (n=6). Differences are significant at * P<0.001 by Student’s t test. ■ untreated, □ with d-penicillamine.
the increased iron accumulation in the kidneys. Thus, our data suggest that renal tubule accumulation of copper is the major cause of spontaneous renal carcinogenesis in LEC rats.

In our study, D-penicillamine treatment increased the hepatic iron concentration and a similar phenomenon has been observed on trientine treatment.\(^\text{39}\) The reason for this is unknown, but the decrease of hepatic copper concentration by chelating agents may increase intestinal iron absorption or iron uptake into hepatocytes.\(^\text{35}\)

Lesions such as altered tubules\(^\text{36}\) and atypical tubules and hyperplasia\(^\text{37}\) are known as renal preneoplastic lesions. In the present study, dilated atypical tubules were observed in LEC rats, and the incidence of these lesions increased with age. Dilated atypical tubules are considered to be preneoplastic lesions, rather than reparative hyperplasia after tubular necrosis. Similar lesions have been observed in the kidneys of Eker rats\(^\text{23}\) and another strain of rats.\(^\text{37}\)

ACKNOWLEDGMENTS

We thank Toshio Yamaguchi, Yoko Ikeda, and Kumiko Fujii for expert technical assistance. This work was supported in part by a Grant-in-Aid (No. 07670250) from the Ministry of Education, Science, and Sports and Culture, Japan.

(Received November 26, 1998/Revised January 29, 1999/ Accepted February 6, 1999)

REFERENCES

1) Li, Y., Togashi, Y., Sato, S., Emoto, T., Kang, J., 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) Sasaki, M., Yoshida, M. C., Kagami, K., Takeichi, N., Kobayashi, H., Dempo, K. and Mori, M. Spontaneous hepatic epithitis in an inbred strain of Long-Evans rats. Rat News Lett., 14, 4–6 (1985).

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

5) 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. (Gann), 79, 828–835 (1988).

6) 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).

7) Kang, J., 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).

8) 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).

9) Leu, M. L., Strickland, G. T. and Gutman, R. A. Renal function in Wilson’s disease: response to penicillamine therapy. Am. J. Med. Sci., 260, 381–398 (1970).

10) Izumi, K., Kitaura, K., Chone, Y., Tate, H., Nakagawa, T., Suzuki, Y. and Matsumoto, K. Spontaneous renal cell tumors in Long-Evans Cinnamon rats. Jpn. J. Cancer Res., 85, 563–566 (1994).

11) Fujii, Y., Shimizu, K., Satoh, M., Fujita, M., Fujioka, Y., Li, Y., Togashi, Y., Takeichi, N. and Nagashima, K. Histochrema demonstration of copper in LEC rat liver. Histochrema, 100, 249–256 (1993).

12) Mohr, U. “International Classification of Rodent Tumours. Part I—The Rat. 3. Urinary System” (1992). IARC Sci. Publ., Lyon.

13) Maekawa, A., Kurokawa, Y., Takahashi, M., Kokubo, T., Ogisu, T., Onodera, H., Tanigawa, H., Ohno, Y., Furukawa, F. and Hayashi, Y. Spontaneous tumors in F-344/DrCj rats. Gann, 74, 365–372 (1983).

14) Haseman, J. K., Arnold, J. and Eustis, S. L. Tumor incidences in Fischer 344 rats: NTP historical data. In “Pathology of the Fischer Rat,” ed. G. A. Boorman, S. L. Eustis, M. L. Elwell, C. A. Montgomery, Jr. and W. F. MacKenzie, pp. 555–564 (1990). Academic Press, San Diego.

15) Chandra, M., Riley, M. G. I. and Johnson, D. E. Spontaneous renal neoplasms in rats. J. Appl. Toxicol., 13, 109–116 (1993).

16) McMartin, D. N., Sahota, P. S., Gunson, D. E., Hsu, H. H. and Spaet, R. H. Neoplasms and related proliferative lesions in control Sprague-Dawley rats from carcinogenicity studies. Historical data and diagnostic considerations. Toxicol. Pathol., 20, 212–225 (1992).

17) Bombard, E. and Rink, M. Frequency of spontaneous tumours in Wistar rats in 2-year studies. Exp. Toxicol. Pathol., 46, 17–29 (1994).

18) Walsh, K. M. and Poteracki, J. Spontaneous neoplasms in control Wistar rats. Fundam. Appl. Toxicol., 22, 65–72 (1994).

19) Goodman, D. G., Ward, J. M., Squire, R. A., Paxton, M. B., Reichardt, W. D., Chu, K. C. and Linhart, M. S. Neoplastic and nonneoplastic lesions in aging Osborne-Mendel rats. Toxicol. Appl. Pharmacol., 55, 433–447 (1980).
20) Yeung, R. S., Xiao, G. H., Jin, F., Lee, W. C., Testa, J. R. and Knudson, A. G. Predisposition to renal carcinoma in the Eker rat is determined by germ-line mutation of the tuberous sclerosis 2 (TSC2) gene. *Proc. Natl. Acad. Sci. USA*, **91**, 11413–11416 (1994).

21) Kobayashi, T., Hirayama, Y., Kobayashi, E., Kubo, Y. and Hino, O. A germine insertion in the tuberous sclerosis (Tsc2) gene gives rise to the Eker rat model of dominantly inherited cancer. *Nat. Genet.*, **9**, 70–74 (1995).

22) Eker, R. and Mossige, J. A dominant gene for renal adenomas in the rat. *Nature*, **189**, 858–859 (1961).

23) Eker, R., Mossige, J., Johannessen, J. V. and Aars, H. Hereditary renal adenomas and adenocarcinomas in rats. *Diagn. Histopathol.*, **4**, 99–110 (1981).

24) Halliwell, B. and Gutteridge, J. M. C. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol.*, **186**, 1–85 (1990).

25) Klein, D., Michaelsen, S., Sato, S., Luz, A., Stampfl, A. and Summer, K. H. Binding of Cu to metallothionein in tissues of the LEC rat with inherited abnormal copper accumulation. *Arch. Toxicol.*, **71**, 340–343 (1997).

26) Sakurai, H., Satoh, H., Hatanaka, A., Sawada, T., Kawano, K., Hagino, T. and Nakajima, K. Unusual generation of hydroxy radicals in hepatic copper-metallothionein of LEC (Long-Evans Cinnamon) rats in the presence of hydrogen peroxide. *Biochem. Biophys. Res. Commun.*, **199**, 313–318 (1994).

27) 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).

28) 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).

29) 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).

30) 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).

31) Suzuki, K., Miyazawa, N., Nakata, T., Seo, H. G., Sugiyama, T. and Taniguchi, N. High copper and iron levels and expression of Mn-superoxide dismutase in mutant rats displaying hereditary hepatitis and hepatoma (LEC rats). *Carcinogenesis*, **14**, 1881–1884 (1993).

32) Kato, J., Kohgo, Y., Sugawara, N., Katsuki, S., Shintani, N., Fujikawa, K., Miyazaki, E., Kobune, M., Takeichi, N. and Niitsu, Y. Abnormal hepatic iron accumulation in LEC rats. *Jpn. J. Cancer Res.*, **84**, 219–222 (1993).

33) Kato, J., Kobune, M., Kohgo, Y., Sugawara, N., Hisai, H., Nakamura, T., Sakamaki, S., Sawada, N. and Niitsu, Y. Hepatic iron deprivation prevents spontaneous development of fulminant hepatitis and liver cancer in Long-Evans Cinnamon rats. *J. Clin. Invest.*, **98**, 923–929 (1996).

34) Toyokuni, S., Tanaka, T., Nishiyama, Y., Okamoto, K., Nakashima, Y., Hamazaki, S., Okada, S. and Hiai, H. Induction of renal cell carcinoma in male Wistar rats treated with cupric nitritotriacetate. *Lab. Invest.*, **75**, 239–248 (1996).

35) Rama, R. and Sánchez, J. Effect of D-penicillamine on iron uptake by isolated rat hepatocytes. *Biol. Trace Elem. Res.*, **18**, 105–113 (1988).

36) Tsuda, H., Moore, M. A., Asamoto, M., Inoue, T., Fukushima, S., Ito, N., Satoh, K., Amelizad, Z. and Oesch, F. Immunohistochemically demonstrated altered expression of cytochrome P-450 molecular forms and epoxide hydrolase in N-ethyl-N-hydroxyethylnitrosamine-induced rat kidney and liver lesions. *Carcinogenesis*, **8**, 711–717 (1987).

37) Dietrich, D. R. and Swenberg, J. A. Preneoplastic lesions in rodent kidney induced spontaneously or by non-genotoxic agents: predictive nature and comparison to lesions induced by genotoxic carcinogens. *Mutat. Res.*, **248**, 239–260 (1991).