A Transplantable Cell Line Derived from Spontaneous Hepatocellular Carcinoma of the Hereditary Hepatitis LEC Rat

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A serially transplantable rat hepatocellular carcinoma (HCC) line was established. The primary spontaneous HCC which developed in a 506-day-old male hereditary hepatitis LEC rat was inoculated into young LEC rats. Only this HCC of 18 primary HCCs was successful in serial transplantation. The established cell line was histologically identical to the primary HCC showing a well-differentiated type with a trabecular structure of tumorous hepatocytes. The characteristic of albumin production was maintained. Chromosome analysis revealed rather widely dispersed polyploid chromosome numbers with a modal value at 96. Every metaphase contained two to five unusually large marker chromosomes.

Key words: LEC rat — Hereditary hepatitis — Hepatocellular carcinoma — Transplantation — Cell line

Spontaneous hepatitis of LEC strain rats was reported previously.^{1,2)} The disease developed suddenly in LEC rats at about four months old, showing typical features of hepatitis: bilirubinuria, hyperbilirubinemia, increased levels of serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and alkaline phosphatase, and histological lesions of the whole liver.^{1,2)} Genetic analysis of LEC rats revealed that a single autosomal recessive gene is responsible for the hepatitis. The recessive gene is designated *hts* (symbol for hepatitis).³⁾ About 30% of rats died of submassive necrosis of hepatocytes, and the remaining animals which survived more than one year developed HCCs later.²⁾ Spontaneous hepatocellular tumors rarely occur in other strains of rats. Therefore, LEC rats are a promising animal model for studies of the association between the pathogenesis of hepatitis and hepatocarcinogenesis. This paper reports the establishment of a transplantable tumor line derived from spontaneous HCC of an LEC rat.

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MATERIALS AND METHODS

Origin and Transplantation of the Primary HCCs in LEC Rats Primary spontaneous HCCs from 18 long-surviving (over 12 months) LEC rats were used for transplantation. Details of the origin and breeding history of the LEC rats were described previously.^{1,2)} The primary HCCs were minced into small pieces and then inoculated subcutaneously or intraperitoneally into one-month-old LEC rats. The recipient rats were sacrificed and examined one month after transplantation. Transplantable tumors obtained were serially inoculated with the same procedure.

Histological Examination All HCCs were examined in histological sections after staining with hematoxylin-eosin (HE).

Detection of Albumin Transplanted tumors were disaggregated in a glass homogenizer. Separated cells were washed three times with phosphate-buffered saline (PBS), suspended in PBS and then dried on slides. After fixation of the cells with 1:1 acetone-methanol, the determination of albumin was performed by the use of fluorescein isothiocyanate (FITC)-conjugated IgG fraction of goat anti-rat albumin serum (Cooper Biochemical, USA). Cells obtained from liver and spleen of a one-month-old LEC rat were used as positive and negative controls, respectively.

Chromosome Analysis For chromosome analysis of transplanted HCC cells, usual air-dried chromo-

some preparations were made. Tumor-bearing recipient rats were treated intraperitoneally with colcemid ($1 \mu\text{g/g}$ body weight) for 1 hr. Tumors were homogenized in a glass homogenizer, filtered through a stainless steel mesh, treated in $0.75M$ KCl for 15 min at 37° , fixed with 3:1 methanol-acetic acid, and air-dried on glass slides. Chromosome preparations were stained with quinacrine mustard and Hoechst 33258 double staining.⁴⁾

RESULTS

Out of 18 primary HCCs (from 5 females and 13 males) inoculated into young LEC rats, only one case of HCC from a male, LSH206G, was successfully developed in the recipients. LSH206G had two distinctive multiple tumors, whitish and reddish nodules (about $5 \times 5 \times 5$ mm) with metastatic lesions in the lung, while most of the nodules in other primary HCCs showed reddish color. One month after transplantation the development of tumors was found in two of four recipients into which whitish nodules of LSH206G were inoculated. Of the two recipients, one developed several small tumors surrounding the uterus. The other showed a widely spread growth of small pieces of tumors ($1 \times 1 \times 1$ mm) over the diaphragm. The small tumors in the latter case were transplanted into four

LEC rats. By contrast, no transplantable tumors were found when reddish nodules of LSH206G were injected into four LEC rats. The liver of LSH206G was 28 g in weight on sacrifice at 506 days of age, while the average weight of the livers of the 18 rats was 26 g (range: 16–52 g) and the average age at sacrifice was 550 days (range: 434–788 days).

In the second passage, all four recipients developed tumors in subcutaneous tissues or mesentery, spreading over the liver, kidney, uterus or diaphragm (Fig. 1). The development of tumors in recipient rats was observed within one month after transplantation as an enlargement of the abdomen. Recipients which were not sacrificed died of large tumors (about $15 \times 15 \times 15$ mm) within two months after transplantation. These large tumors often showed a hemorrhagic tendency. At

Fig. 1. Gross appearance of the abdominal cavity of a recipient rat 24 days after the fifth passage of HCC cells. Tumors of various sizes are developing in subcutaneous tissues and the mesentery (arrows).

Fig. 2. The primary HCC, LSH206G, showing a well-differentiated type with a trabecular structure of tumorous hepatocytes (HE, $\times 200$).

Fig. 3. Transplanted tumor in a syngeneic rat at the fifth passage, exhibiting histologically identical features to the primary HCC (Fig. 2) (HE, $\times 200$).

Fig. 4. Immunofluorescence staining of albumin in transplatable HCC cells from the fifth passage, showing a positive reaction in the cytoplasm ($\times 840$).

present, the tumors have been maintained in serial transplantation to the seventh passage.

Histologically, transplanted tumors were identical to the primary HCC showing a well-differentiated type with a trabecular structure of tumorous hepatocytes (Figs. 2 and 3). The cells had basophilic cytoplasm and round nuclei with clear nucleoli both in the primary and transplanted tumors. The remaining 17 primary HCCs were also of well-differentiated type. However, tumor cells of whitish nodules in LSH206G had more basophilic cytoplasm, a higher nucleus-cytoplasm ratio and more mitoses than those of reddish ones.

Intracellular albumin was clearly detected as a fluorescent substance in the cytoplasm of transplatable hepatocytes (Fig. 4). As expected, such positive immunofluorescence was observed in normal hepatocytes of a young LEC rat, while spleen cells exhibited a negative reaction.

Chromosome analysis was carried out in the seventh-passage transplanted cells. Chromosome numbers were widely distributed between 21 and 105 with a modal value at 96 (Fig. 5). No diploid metaphase was observed. Every metaphase had two to five large re-

Fig. 5. Frequency distribution of chromosome numbers in 60 metaphases of the transplatable HCC cells from the seventh passage.

Fig. 6. A metaphase spread from the transplatable HCC cells of the fifth passage, followed by Q-band staining ($\times 700$). Arrows indicate large marker chromosomes.

arranged marker chromosomes (Fig. 6). The origins of these marker chromosomes were not precisely traceable, but their Q-band patterns suggested that they should be translocation products involving chromosomes 1, 2 or 3. A metaphase having 21 chromosomes also contained one marker chromosome.

DISCUSSION

The transplatable rat cell line from spontaneous HCC described here is the first to be reported in the literature, although a number of liver tumor cell lines have been established from chemically induced HCCs of laboratory animals. Hepatocarcinogenesis related to hepadnavirus was described in human,^{5,6)}

woodchuck,⁷⁾ ground squirrel⁸⁾ and Pekin duck,⁹⁾ and among these animal HCCs, only a woodchuck transplantable tumor line was established.^{10, 11)} In LEC rats no hepatitis virus was detected, and inheritance of the disease is simple autosomal recessive.^{2, 3)} Therefore, the present transplantable cell line provides a novel material for further analysis of hepatocarcinogenesis as well as the pathogenic mechanisms of hereditary hepatitis in LEC rats.

Since the discovery of hereditary hepatitis in LEC rats, we have followed the natural history after the onset of hepatitis and observed the development of HCCs in a very high incidence (more than 90%) among long-surviving animals (over 12 months old). Details of the high incidence and pathological features of HCCs in LEC rats will be described elsewhere. In the present study, the primary HCCs of 18 long-surviving LEC rats were inoculated into syngeneic rats, and only a tumor line from one male, LSH206G, could be successively transplanted. In respect to liver weight and age, LSH206G showed no remarkable difference from other primary HCCs. However, in the primary LSH206G, lung metastasis was found, and the liver had more whitish nodules consisting of tumor cells with more basophilic cytoplasm and a higher nucleus-cytoplasm ratio and more mitoses of actively developing tumor cells. Thus, this HCC seems to be more malignant than the others. The degrees of malignancy of the primary HCCs in LEC rats examined here appeared to be different from each other, as judged from transplantation results and histological examinations.

Chromosome analysis of this line revealed widely spread polyploidy ranging from mostly tetra- to pentaploid. This might be concordant with the fact that the transplantable tumors consist of histologically large hepatocytes with round nuclei, like the primary HCC. Albumin production as one of the main hepatic functions remained in the cell line. The large marker chromosomes were consistently present in every metaphase, although their precise origin was not traceable. Our preliminary examination of *in situ* hybridization to the present transplantable tumor metaphases showed no clustering of silver grains at *N-myc*, *N-ras*, *H-ras*, or placental glutathione S-transferase (GST-P) genes on these marker

chromosomes, suggesting no remarkable amplification of these genes in the present cell line. Activity of the GST-P gene is known to be associated with chemical hepatocarcinogenesis.¹²⁾ *N-myc*,^{13, 14)} *N-ras*,¹⁵⁾ and *H-ras*¹⁶⁾ are often amplified in cell lines from solid tumors.

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