TRANSPLANTABLE CHORIOCARCINOMA OF RATS INDUCED BY FETECTOMY AND ITS BIOLOGICAL ACTIVITIES

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Eight cases of choriocarcinoma were induced in WKA female rats by simple fetectomy without viral inoculation or administration of chemical carcinogens. Among them, one line has been successfully subcutaneously transplanted over 10 generations in syngeneic rats and this line was designated as RCHO. The histological appearance of RCHO consisted of trophoblastic giant cells and small basophilic tumor cells, which resembled normal rat placenta. RCHO possessed marked mammotrophic and lactogenic activities in female recipients and induced atrophy of the testes in male recipients.

Key words: Rat — Choriocarcinoma — Transplantation — Fetectomy — Placental lactogen

Choriocarcinoma of rats is a rare tumor and resembles human choriocarcinoma with regard to histological features and biological activities.1-6,9,10) After Shintani et al.9) reported for the first time in 1966 the induction of choriocarcinoma in rats by substituting 7,12-dimethylbenz[a]anthracene (DMBA)-impregnated wax pellets for fetuses, the administration of chemical carcinogens to pregnant rats was reported to induce the tumor.1-6,10)

On the other hand, in 1976 Sakashita et al.7) succeeded in inducing malignant tumors in pregnant rats by fetectomy without administration of carcinogens or viral inoculation. By the same method as reported previously,11) we also induced yolk sac carcinomas, adenocarcinomas and teratomas in a total of 103 rats out of 127 operated rats. In nine of these rats, small foci of atypical trophoblastic cells could be seen within the other type of tumor.

In this experiment, choriocarcinoma was induced in eight WKA rats by the same method, and the tumor became transplantable in syngeneic rats. This is the first report concerning dysontogenetically induced transplantable choriocarcinoma that has kept its biological activities, such as mammotrophic and lactogenic effects, in every transplantation generation.

MATERIALS AND METHODS

Eighty-three female WKA rats were used for the induction of tumors. These rats were obtained from the Institute for Animal Experiments, Hokkaido University School of Medicine, and were bred with brother-sister mating in our laboratories. After female WKA rats had been mated with syngeneic males, they were fetectomized on day 12 of gestation. The details of the procedure were described by Sakashita et al.7,8) Rats used for serial transplantation were 3-week-old weanling WKA rats of both sexes. At each transplantation, portions of the removed tumor were minced into pieces smaller than 1 mm³ and inoculated in the subcutaneous tissue of the lateral chest wall. Ovaries, mammary glands and uteri of females, testes of males and pituitary glands of both sexes were examined histologically and compared with those of control rats of the same age.
RESULTS

Out of the 83 rats operated upon, 59 developed tumors. They had one to several tumor nodules at the uterine wall, and some nodules adhered to each other, resulting in tumors as large as $6 \times 5 \times 5 \text{ cm}^3$ after 6 months. The tumors were divided into four types according to their histological appearance, and consisted of 39 yolk sac tumors, 24 adenocarcinomas, 30 teratomas and 8 choriocarcinomas. These tumor types were mixed in one nodule, in which dark red areas histologically showing choriocarcinoma were readily apparent. Histologically, two characteristic types of cells were observed in choriocarcinoma, i.e. small basophilic tumor cells and large mono- or multinucleated tumor cells (Fig. 1). The former resembled small cytotrophoblasts of the rat placenta. The latter were irregular, large, often huge, bizarre cells with a large amount of cytoplasm, whose pseudopodia extended between other cells. Their deeply stained nuclei were large with distinct nucleoli. They were suggestive of trophoblastic giant cells or syncytiotrophoblasts.

Primary tumors were easily transplanted to syngeneic rats subcutaneously. However, almost all the lines consisted of adenocarcinoma or yolk sac carcinoma, and a few lines consisted of small basophilic tumor cells, which could not be considered choriocarcinoma. Only one line of pure choriocarcinoma, named RCHO, was established and passaged subcutaneously for more than ten generations (Fig. 2). RCHO was dark red, and histologically consisted of trophoblastic giant cells, small basophilic tumor cells and cells with histological transition between the two. The trophoblastic giant cells were arranged in sheets, and formed the major proportion of the sinus-like structure with little intervening stroma or few

![Fig. 1. Choriocarcinoma. Trophoblastic giant cells are arranged in sheets. Small basophilic tumor cells are seen on the lower right. H-E. × 170.](image)
Fig. 2. Transplanted choriocarcinoma (RCHO) is grossly dark red and hemorrhagic.

Fig. 3. RCHO is composed of trophoblastic giant cells and small basophilic cells. H-E. ×170.
Fig. 4. Breast of a recipient (right) and a control rat (left). Both are 7-week-old siblings.

Fig. 5a. Breast of a control rat rich in fat tissue. H-E. ×170.
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Capillaries (Fig. 3). Viable tumor cells existed at the peripheral part of the tumor surrounding large areas of central necrosis and hemorrhages. No component of adenocarcinoma or yolk sac carcinoma was observed in any transplantation generation of RCHO.

Quite remarkable mammotropic and lactogenic effects were observed in female recipients of RCHO. After transplantation of RCHO, abdominal mammary glands grew rapidly, reaching 4 times the size of those of control rats (Fig. 4). Marked epithelial proliferation occurred, with a great increase in the number and the size of the acini. The cells lining the dilated acini showed secretory activity with pale cytoplasm and globules (Fig. 5). However, no decidual changes were observed in the uterus of the recipients, and no luteal cell hypertrophy was seen in the ovary. Atrophy was observed in the testes of male recipients. The mean weight of the testis 4 weeks after transplantation (7-week-old rats) was 73 mg, while that of control siblings was 740 mg (Fig. 6). The seminiferous tubules became smaller and were situated far apart. Spermatogonia and germinal epithelial cells disappeared, leaving only Sertoli cells resting on a distinct basement membrane. Interstitial tissue was edematous, and Leidig cells were neither hyperplastic nor hypertrophic (Fig. 7) Pituitary glands of the recipients showed unremarkable histological changes.

DISCUSSION

Biological activities of choriocarcinoma induced by administration of chemical carcinogens were studied by Shintani et al.9) and Tanaka.10) Shintani et al. demonstrated that rats injected with tumor homogenates showed mammotropic activity in the lobuloalveolar growth of the mammary gland. Tanaka showed that intraperitoneal injection of incubation medium of tumor tissue had luteotropic or FSH-like activity. In this study, every transplantation generation of RCHO had mammotropic and lactogenic...
Fig. 6. Testes of a recipient (right) and a control rat (left). Both are 7-week-old siblings. Kidneys of the recipient are as large as those of the control rats.

Fig. 7a. Testis of a control rat showing active spermatogenesis. H-E. ×170.
activities, which resembled the activity of the tumors induced by Shintani et al.

The biological activities of RCHO seem to be due to increased rat placental lactogen, although this is not certain. On the other hand, the average values of serum estrogen and progesterone in four female recipients at 4 weeks after transplantation were lower than those of control siblings: $E_1$ decreased from 270 pg/ml to 172 pg/ml, $E_2$ from 35 pg/ml to 27 pg/ml, and progesterone from 10 ng/ml to 5 ng/ml. The value of prolactin varied from 41 ng/ml to 48 ng/ml. (These serum levels were determined by radioimmunoassay at Teikoku Zoki Ltd., Kawasaki.)

The value of this experiment lies in the fact that true choriocarcinoma could be induced dysontogenetically without administration of chemical carcinogens or inoculation of viruses. We suggested in an earlier paper that tumors induced by fetectomy could arise from primordial germ cells.

Choriocarcinoma might be of the same origin. Some microenvironmental influences on germ cells may be changed by fetectomy, so that different types of phenotypic expression may occur in the tumor, despite the common histogenetic origin.

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Fig. 7b. Testis of a recipient rat showing atrophy of seminiferous tubules. H-E. ×170.