Growth and Differentiation of Teratocarcinoma OTT6050 Affected by Host Hormonal Changes in Old Mice

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KUBOTA, K., KUBOTA, R. and MATSUZAWA, T. Growth and Differentiation of Teratocarcinoma OTT6050 Affected by Host Hormonal Changes in Old Mice. Tohoku J. exp. Med., 1987, 151 (3), 253-259 — We previously reported the age and sex dependent decrease in the growth rate of teratocarcinoma OTT6050. Studies of the effects of hormones on the tumor growth are presented here. Age related changes in the serum levels of triiodothyronine, thyroxine, progesterone, testosterone and estradiol that increase in the young adult and decrease in the old were observed with radioimmunoassay. Administration of estradiol to tumor-bearing, 50 week-old female mice increased the tumor growth rate to that of 10 week-old mice. Tumor growth rates in estradiol-treated 10 week-old female and male mice were slightly accelerated compared to the control, but not as fast as that of control juveniles. It can be concluded that extrinsic estradiol stimulates the teratocarcinoma growth in vivo especially in old female mice. Age-dependent decline of the growth rate of teratocarcinoma in the female is due partly to changes in estrogen level. —— teratocarcinoma OTT6050; tumor growth; serum hormone level

Since most cancers occur in middle and old age, aging in host must be considered to be a very important factor in the study of the host-tumor interaction, for the management of cancer patients, and for the understanding of geriatric medicine.

The mouse teratocarcinoma, which can differentiate to a wide range of tissue types, is a unique model for the study of neoplastic growth and differentiation (Pierce 1967; Stevens 1970). We reported previously (Kubota et al. 1981) the effect of age and sex of syngeneic host mice on the growth rate and differentiation of teratocarcinoma OTT6050. Tumor growth rate is very fast in the juvenile hosts (2 and 3 week-old) and decreases in female hosts with age. But in the male, tumor growth is constant from 10 to 70 week-old (w.o.). A strong correlation between the growth rate and the degree of histological differentiation was observed. We suggested a possible mechanism, namely age-related changes in some hormone levels, especially estrogen, might influence the tumor growth rate.

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In the present study, the effect of hormones on the tumor growth is described in order to test the hypothesis that age-dependent changes of tumor growth might be caused by hormone levels.

**Materials and Methods**

**Tumor and mice**

Mouse teratocarcinoma OTT6050 (Stevens 1970) and syngeneic 129/Sv-CP+Sl' mice were used in this study. A suspension of embryoid bodies, the ascitic form of teratocarcinoma, was prepared as described previously (Kubota et al. 1981). 0.1 ml of cell suspension (8 x 10^7 cells/ml) was injected subcutaneously into the backs of 129/Sv mice.

**Tumor growth curve**

Solid tumors on the backs of mice were measured with a vernier caliper. The logarithm of the product of height, width and length of the tumor (tumor volume) was plotted versus time (Kubota et al. 1981).

**Hormone level measurements**

Juvenile (2-3 w.o.), young adult (10 w.o.), and old (50 w.o.) male and female mice were used. A mouse was decapitated between 9 and 10 a.m., as soon as possible after removal from the cage. Trunk blood was collected and the serum was stored at -20°C until radioimmunoassay was carried out. Serum testosterone, progesterone, estradiol, triiodothyronine, thyroxin and insulin were measured by radioimmunoassay using commercially available assay kits (Eiken Chemical Labo., Tokyo, Daiichi RI Labo., Tokyo, Dainabot RI Labo., Tokyo).

**Administration of estradiol**

A group of seven old female (50 w.o.) mice bearing solid teratocarcinomas on the back received an intraperitoneal injection of benzoate aqueous emulsion of 0.05 mg of estradiol (Teikoku Hormone Mfg. Co., Tokyo) three times per week. The injections were started when the “tumor volume” exceeded 100 cu mm (e.g., tumor diameters of 5, 5, and 4 mm). The day tumor volume exceeded 100 mm³ was designated as Day 0. Tumor volume was measured six times per week. Survival times of the mice from Day 0 were noted. Autopsies recorded the weights of spleen, liver and uterus, and the excised tumors were fixed, sectioned and stained with hematoxyline and eosine for histological examination.

**Results**

**Control growth curves**

Age-related changes of tumor growth rate were already described by us (Kubota et al. 1981). Some of these data were used in this study as the control group. Fig. 1 shows that tumor growth curves of both male and female mice of 2, 3, 10, 30, 50 and 70 w.o. on log-linear graphs. In the 2 and 3 w.o. of both sexes, tumors grew fastest. In the male mice from 10 to 70 w.o., there were no significant changes in tumor growth rate, but tumors grew slower than in 2 and 3 w.o. So, tumor growth curves were apparently divided into two phases, juvenile (2 and 3 w.o.) and adult (from 10 to 70 w.o.) (p < 0.01). On the other hand, in the female mice, tumor growth rate slowed from 10 to 70 w.o. Then, tumor growth curves were divided into three phases, juvenile, young adult (p < 0.05 compared with
Hormone level measurements

To study age-related changes of host factors that influence tumor growth, some serum hormones were measured. The age-related three-phase pattern (low level in juvenile, high in young adult, and low in old mice) was observed for triiodothyronine, thyroxine, progesterone, testosterone in male and for estradiol in juvenile) and old ($p < 0.05$, compared with young adult).

Fig. 1. Control tumor growth curves by age and sex.
Abscissa: Time (days) after tumor volume exceeded 100 cu mm; Ordinate: Tumor volume (product of three diameters) plotted on log scale.
female. Triiodothyronine and thyroxine were significantly low in the old female group (Fig. 2). Others showed no significant change with aging.

**Effects of estradiol**

Triiodothyronine, testosterone and estradiol were given to old male and female mice bearing solid tumors to study the age-dependency of tumor growth and its relation to these hormones. Although triiodothyronine and testosterone did not affect the tumor growth of old mice (data not shown), estradiol accelerated the teratocarcinoma growth of old female mice. To determine the effect of treatment and compare it with the control curve, tumor volumes of the mice were measured everyday and then those volumes for 5 consecutive days were averaged in each group (Fig. 3). The tumor growth rate of estrogen-treated 50 w.o. females was significantly faster than that of control 50 w.o. females and was similar to that of 10 w.o. young females. The survival time of estradiol-treated mice (44.0±7.5 days) was not different from that of the control group (49.0±13.0 days).

The weight of uteri of the estradiol-treated mice was significantly heavier than the controls or the normals (Table). Drug-induced liver damage was suspected because of the marked liver enlargement. Although the tumor growth rate of the treated group at its logarithmic phase was faster than that of controls, the tumor weights at death were less ($p < 0.05$) than those of controls.
There was no difference in the degree of histological differentiation of the tumors between the estradiol-treated mice and the controls. The tumors contained large necrotic areas, but there were various kinds of well differentiated tissues around the necrosis. These include neural tubules, epithelial tissues ciliated, non-ciliated or keratinized, hyaline cartilage, bone and skeletal muscle. Small clumps of embryonal carcinoma cells were observed among the differentiated tissues and in the peripheral area of the tumor.

**DISCUSSION**

It is generally known that there is a decline in testicular, ovarian, and thyroid function in old age. Human studies show a highly significant decrease in triiodothyronine with age (Snyder and Utiger 1972; Rubenstein et al. 1973). Plasma testosterone levels are lower in 12 month-old rats than at 5 months (Bethea and Walker 1979). Plasma estradiol levels are significantly lower in mice aged 12-15 months than 3-5 months (Parkening et al. 1978). Age-related changes of

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**TABLE 1.** Weights (g) of spleens, livers, uteri and tumors of 50-week-old female mice

<table>
<thead>
<tr>
<th></th>
<th>Spleen</th>
<th>Liver</th>
<th>Uterus</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.117±0.040</td>
<td>1.147±0.077</td>
<td>0.271±0.031</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>0.329±0.039</td>
<td>1.623±0.146</td>
<td>0.236±0.043</td>
<td>29.7±9.6</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.392±0.153</td>
<td>2.351±0.673*</td>
<td>0.617±0.073**</td>
<td>20.8±4.3*</td>
</tr>
</tbody>
</table>

Estradiol (0.05 mg) was given intraperitoneally thrice weekly. Values are means±s.d.

* p < 0.05 (Student’s t-test), as compared with control.
** p < 0.01 (Student’s t-test), as compared with control.
hormone levels observed here in 129/Sv mice are consistent with these previous studies. Loss of reproductive capacity among 129/Sv mice at 10-12 m.o. is also observed in this study.

Age-related changes of thyroid hormone and sex hormone levels may interact and together affect tumor growth rate. In order to test this hypothesis we administered these hormones to the tumor-bearing mice. Administration of only estradiol to the old (50 w.o.) female mice increased the tumor growth rate to approximately that of 10 w.o. young mice. The tumor growth rate of 10 w.o. female and male mice treated with estradiol were slightly accelerated compared to the control, but not so fast as that of 3 w.o. (Kubota et al. 1983).

It can be concluded that extrinsic estradiol accelerates teratocarcinoma growth in vivo especially in old female mice. Age-dependent decline of growth rate of teratocarcinoma in the females is partly due to the changes in estrogen level.

Endocrine dependency of certain tumors has been well documented particularly with respect to sex hormones (breast cancer, prostatic cancer). Recently not only the tumors of breast and uterus, but also tumors of colon, rectum, lung and kidney were proved to contain binding sites for estradiol, even in male patients (Maillot et al. 1980). Characteristic sex steroid metabolism has been reported in cultured pluripotent teratocarcinoma cells (Antila and Wartiovaara 1980). The specific binding of estradiol in fetal testes increased during gestation (Pasqualini et al. 1980). Therefore, it is a reasonable hypothesis that there may be some interaction between estrogen and teratocarcinoma. The stimulating effect of estradiol on teratocarcinoma growth was observed here, and age-dependent decline of tumor growth rate in the female could be explained by the changes of estrogen level. In addition, we thought the increase in the weight of uteri of the estradiol-treated mice was due to its direct effect.

Two possibilities remain to be explored: whether the tumor growth is influenced by the estradiol indirectly through the host metabolic changes, or directly through the specific binding of estradiol to the teratocarcinoma. These are further problems to be studied. We believe that studying tumor environmental factors in vivo will lead to a better understanding of host-tumor relationships and effective treatment of cancer patients.

Acknowledgments

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