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Relapse of Androgen-dependent Tumour of Mouse (Shionogi Carcinoma 115) after Castration and Oestrogen Treatment

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Summary. To elucidate relapse of hormone-dependent tumour, mice bearing androgen-dependent carcinoma (SC 115) underwent castration or received oestrogen treatment. Since SC 115 consisted of two types of cells, androgen-sensitive round cells and -insensitive spindle-shaped cells, changes in the ratio of the cellular population were examined. After castration, spindle-shaped cells increased temporarily as the tumour regressed, then the round cells increased significantly along with an increase in size of the tumour. Oestrogen treatment did not influence the population. Androgen dependency and the growth rate of round cells were generally preserved in the next generation. Therefore, relapse may occur by an increase in the number of androgen-sensitive round cells.

Key words: Androgen-dependent tumour, Relapse, Shionogi carcinoma (SC 115), Hormone dependency of tumour.

Introduction

It is well known that relapse of prostatic cancer occurs after initial suppression of its growth by deprivation of androgen. Reasons for the relapse might be explained by pre-existing androgen-insensitive tumour cells or by mutation to form independent cells, but the mechanism is still obscure [4]. An androgen-dependent mammary gland tumour of mice (SC 115, Shionogi Carcinoma 115) was established and it has been generally accepted that growth of this tumour requires androgen for its initial growth [11, 12]. After the tumour reaches a certain size, however, castration causes temporary regression, followed by an increase in the size of the tumour [8]. Androgen receptors and nuclear uptake of androgen were observed in the SC 115 [1, 5, 6, 10, 13, 15, 17, 18, 21]. From this evidence the SC 115 seems to be an appropriate model for human prostatic cancer. The SC 115 is an undifferentiated medullary adenocarcinoma consisting of round cells, but it has been recently reported that after deprivation of androgen spindle-shaped cells appear in tissues of the SC 115. The spindle-shaped cells do not contain androgen receptors, suggesting that they are independent of androgen [8]. However, the role of the spindle-shaped cells on relapse seems to be obscure. The present study was undertaken to examine changes of cellular population and properties of the constituent cells on relapse of the SC 115.

Materials and Methods

Animals

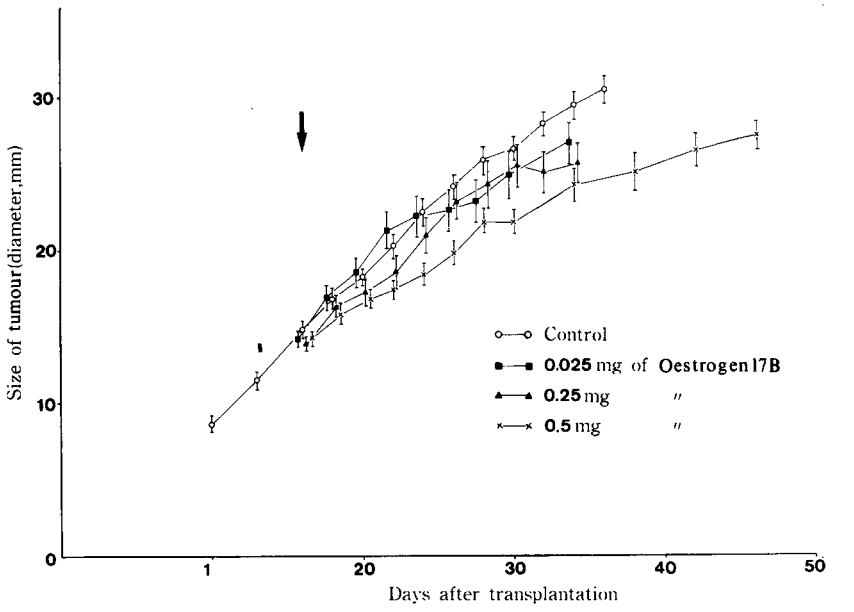
Male mice of DD/S strain 5–6 weeks old and weighing approximately 23 g at the time of transplantation of tumours were used throughout the experiments. This strain was originally supplied from the Shionogi Research Laboratory, Osaka, Japan. Castration was performed on the 16th day after tumour inoculation. Except where otherwise indicated, oestrogen treatment was performed by injection of a daily dose of 0.5 mg of oestradiol-17β benzoate dissolved in 0.1 ml of sesame oil per animal between 16–20 days after tumour implantation. As controls, animals received the same amount of sesame oil daily during the same period.

Tumours

SC 115 was supplied from the Shionogi Research Laboratory and has been maintained in our laboratory [20]. Tumours between the 190th and 207th generations were used. A piece of tumour, approximately 5 mg in wet weight (approximately 10^7 cells) was implanted subcutaneously with a trocar in the dorso-median region of the neck of recipient mice.

Measurement of Tumour Size and Doubling Time

Tumours were palpable by about the 8th day after implantation. Thereafter, the length and width of the individual tumours were measured and the mean of length and width referred to as the tumour size. Doubling time was calculated from changes of tumour volume during exponentially growing periods [16].



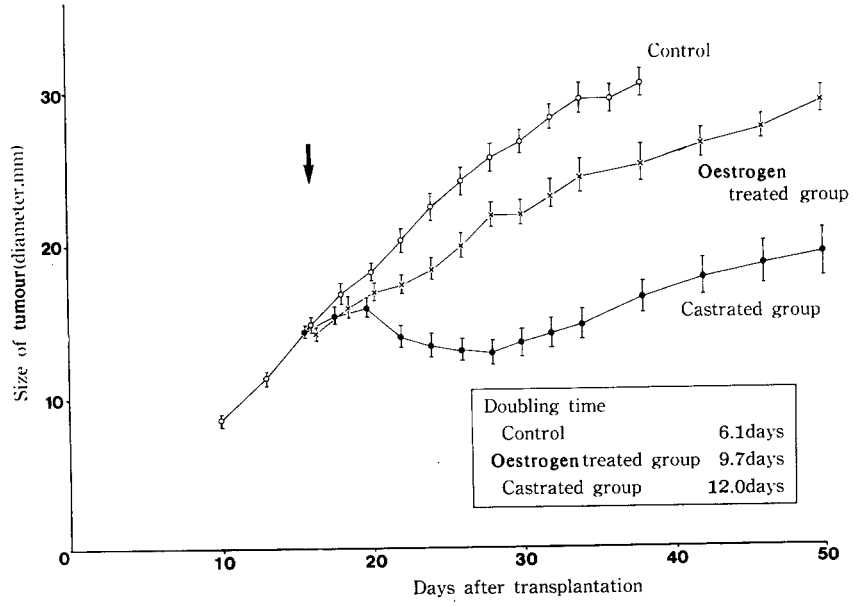


Fig. 1. Effect of oestrogen on growth of SC 115. Fifty animals bearing SC 115 were divided into four groups; control, daily doses of 0.025 mg, 0.25 mg and 0.5 mg of oestradiol-17β benzoate-injected groups. Administration of hormone was started on the 16 th day after tumour implantation (*arrow*) and was continued for 5 days. Differences between control and 0.5 mg-injected group after 20th day of tumour implantation were statistically significant ($p < 0.05$)

Fig. 2. Effects of castration and of oestrogen treatment on growth of SC 115. Forty-five animals bearing SC 115 were divided into three groups on 16th day after tumour implantation (*arrow*): one retained as control, and the others were subjected to either castration or oestrogen treatment. Animals in oestrogen-treated group received 0.5 mg of oestradiol-17β benzoate for five consecutive days. Differences between control and castrated group after 18th day ($p < 0.05$) and 20th day ($p < 0.01$) transplanted, between control and oestrogen-treated group after 20th day ($p < 0.05$) and 22nd day ($p < 0.01$), and between castrated group and oestrogen-treated one after 20th day ($p < 0.01$) were statistically significant. Mean survival days after implantation were as follows: control; 46.7 ± 2.6, castrated group; 70.5 ± 3.4 except three mice surviving over 90 days after implantation, oestrogen-treated group; 55.3 ± 2.8. Statistically significant differences between control and castrated group ($p < 0.01$), and between control and oestrogen-treated group ($p < 0.05$) *Insert:* Doubling time was obtained from changes of tumour volumes during linearly growing periods (16th–36th day of control, 18th–42nd day of oestrogen-treated and 30th–50th day of castrated animals) [16]

Estimation of Ratio of Cellular Population in Tumour Tissue

Tumours were removed on the day indicated. They were oval in shape and encapsulated with thin fibrous tissue. Each tumour was cut into slices 2 mm in thickness and stained by haematoxylin-eosin and Azan-Mallory methods. Areas in the section were divided into three parts; round cells, spindle-shaped cells, and necrosis with fibrous connective tissue.

Results

Effect of Dose of Oestrogen on Tumour Growth

Daily doses of 0.025 mg, 0.25 mg or 0.5 mg of oestradiol-17β benzoate were injected between the 16th and 20th day after tumour implantation, and the effect of these doses of oestrogen on tumour growth was examined (Fig. 1). Tumours in oestrogen-treated mice showed a retarded growth curve. Since a significant effect was obtained only after treatment of animals with relatively large amounts of oestrogen, subsequent experiments of oestrogen treatment were performed using 0.5 mg of oestradiol-17β benzoate.

Effects of Castration and of Oestrogen Treatment on Tumour Growth

After castration the tumour growth ceased and tumours regressed temporarily, then growth resumed. But the growth rate of tumour on relapse was significantly lower than that of the original SC 115. Oestrogen treatment also caused retarded tumour growth but was less effective than castration (Fig. 2). The retarded tumour growth observed in the treated groups was reflected in the survival of the animals (Fig. 2 legend).

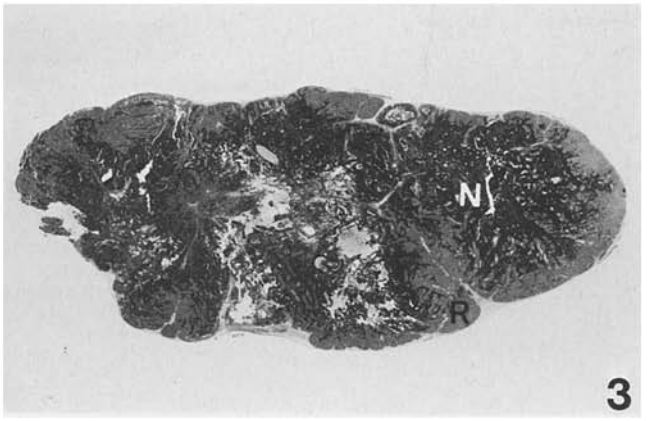


Fig. 3. A low-power view of tumour (SC 115, control group, 20 days after tumour implantation), showing proliferation of undifferentiated medullary carcinoma consisting of round cells (*R*). Note extensive necrosis largely confined to central portion (*N*)

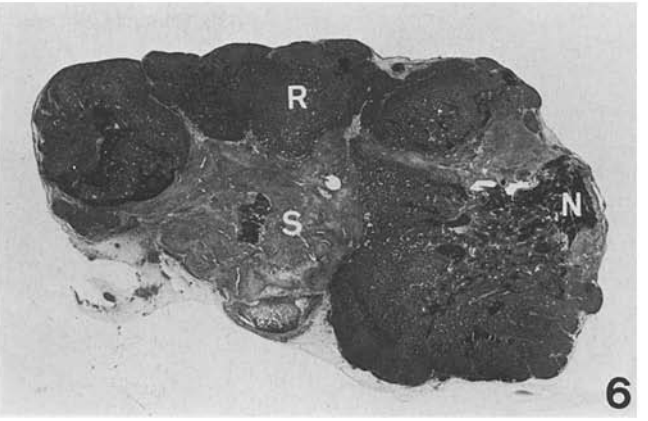


Fig. 6. A low-power view of tumour (castrated group, 40 days after tumour implantation), showing scattered foci of necrosis (*N*), massive proliferation of round cells (*R*) and spindle-shaped cells (*S*). Note intermingled cellular pattern of round and spindle-shaped cells

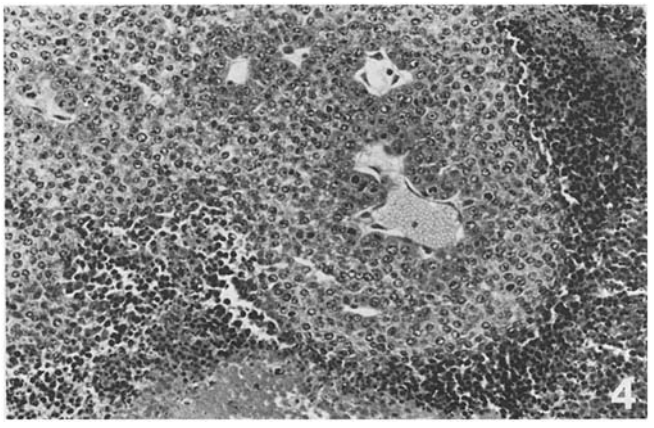
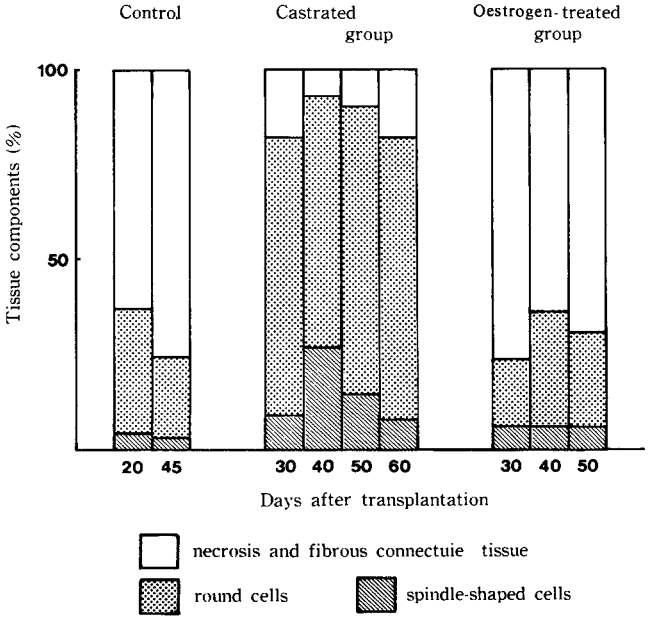


Fig. 4. A high-power view of tumour (same as Fig. 3), showing solid proliferation of undifferentiated round cells with massive necrosis



◀ Fig. 5. Ratios of cellular population in tumour tissues. Tumours removed from ten tumour-bearing mice were used for depiction of each column, and ratio of cellular population in the tumour tissue was calculated

Effects of Castration and of Oestrogen Treatment on Cellular Population of the Tumour

Tissues of the SC 115 consisted of round cells and large necrotic areas (Figs. 3 and 4). Moreover, among the round cells, a small number of spindle-shaped cells was observed in the centre of the tumour nodules. The spindle-shaped cells contained slender nuclei; however, some spindle-shaped cells adjacent to the round cells included round nuclei suggesting transformation from the round cells. The ratio of necrosis to the whole tumour was increased with increase of tumour size (Fig. 5).

As the size of the tumour decreased after castration, areas of necrosis in the tumour were significantly decreased with concomitant increase in the ratios of the spindle-shaped cells, and to a lesser extent, of the round cells to whole tumours (Figs. 6 and 7). Thereafter, the population of the spindle-shaped cells increased gradually. However, by the 50th day after tumour implantation, when growth of the tumours was restored, the ratio of the spindle-shaped cells to the whole tumour decreased with concomitant increase in the ratio of round cells. This suggests that the tumour growth on relapse after castration was mainly attributable to an increase in round cells, although the rate of cell division of these cells was less than that of the original SC 115 grown in the control animals (Table 1). The mitotic index of the spindle-shaped cells was not changed during the observation period, indicating that proliferation of these cells was independent of the level of androgen. Oestrogen treatment did not alter the cellular population in the tissue.

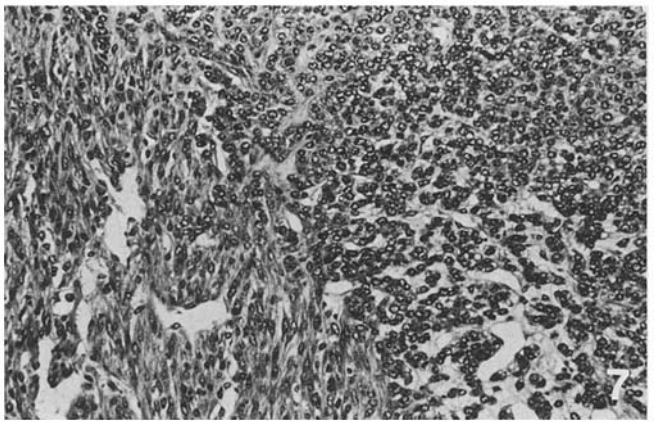


Fig. 7. A high-power view of tumour (same as Fig. 6), showing solid proliferation of tumour cells consisting of round cells and spindle-shaped cells

Table 1. Mitotic index[a] of tumours removed from control, castrated, and oestrogen-treated animals

Source of tumour	Days after tumour transplantation			
	20th	30th	40th	50th
Control	11.2			
Castrated mice				
Round cells		7.8	7.9	8.5
Spindle-shaped cells		3.1	3.1	2.9
Oestrogen-treated mice		10.8	11.2	10.9

[a] Mitotic index is expressed as number of mitotic cells/1,000 tumor cells. Mitotic index of tumors removed from control and oestrogen-treated animals is obtained from dividing and non-dividing round cells

Transplantation of Tumours Removed from Castrated and Oestrogen-Treated Animals

After the tumours reached more than 2 cm in size, they were transplanted to male and female mice. The tumour tissues from the castrated animals consisted of hard and soft parts; the main constituents of the former and of the latter were the spindle-shaped cells and the round cells with necrosis, respectively, and these two parts were transplanted separately.

Tissues obtained from the control animals were not grown in female animals, showing characteristics of the original SC 115. However, some tumour tissues obtained from the castrated and from the oestrogen-treated animals were successfully transplanted to female mice, though the rate of successful transplantion was relatively low (Fig. 8 legend). Growth of these tumours in the recipient mice is shown in Fig. 8. Transplants consisting of round cells grew as rapidly as the original SC 115. However, growth of the transplants composed of the spindle-shaped cells was relatively slow. Growth rates of the transplants taken from

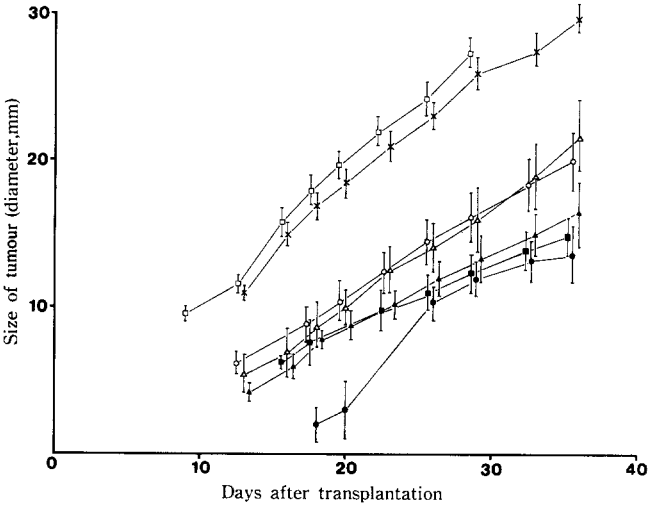


Fig. 8. Growth of transplanted tumours removed from the control, castrated, and oestrogen-treated animals. Tumours removed from the experimental animals were implanted to male and female recipient mice of DD/S strain. Tumours from the castrated animals were separated into soft and hard parts, and each was served as transplants. The following numbers of numerator and those of denominator are numbers of tumour grown and those of recipient mice, respectively. Tumour from control mice to male recipient (×, 10/10), Soft part of tumour from castrated mice to male (□, 10/10) and female (■, 3/10), Hard part of tumour from castrated mice to male (△, 10/10) and female (▲, 5/10), and tumour from oestrogen-treated mice to male (○, 10/10 and female (●, 3/10)

any tumour were similar to each other in female mice but were significantly lower than growth rates observed in male animals. These results indicated that androgen dependency and growth rate of tumours were generally preserved in the next generation after growing under androgen-deprived conditions.

Discussion

The present study shows that the administration of relatively large amounts of oestrogen was necessary for induction of changes in tumour growth. Reasons for the requirement of such unphysiologically high doses of oestrogen are not clear; however, mice of the strain used in the present study may be relatively insensitive to oestrogen [3]. Alternatively, injection of huge amounts of oestrogen were not as effective as was expected, due to an increase in the weight and androgen secretion of the adrenal gland, and this may compete with the biological effect of oestrogen [19].

It was reported that deprivation of androgen caused the appearance of spindle-shaped cells [8]. The main abnormality in chromosomes was almost equally shared in the spindle-shaped and the round cells, therefore, the spindle-shaped cells were assumed to be derived from the round cells [7]. In the present experiments, transformation of the round cells to spindle-shaped cells was assumed histologically. Although the amount of the spindle-shaped cells was small,

these cells were also observed in the tumours grown in the control as well as in the oestrogen-treated animals. Therefore, the observation of abundant spindle-shaped cells after castration is probably due to an acceleration of the transformation in this period. The spindle-shaped cells were usually found in the central part adjacent to necrosis. Since in this site the blood supply seems to be diminished, transformation of the round cells to spindle-shaped cells might take place under conditions lacking hormones or nutritions. This might explain the androgen independency of the spindle-shaped cells. Although some of these cells might be transformed from round cells, most of the increase occurred as the result of cell division. On the contrary, transformation of the SC 115 cells to spindle-shaped cells was observed when grown in culture media containing a high level of androgen [2, 22]. Differences in cell shapes between in vivo and in vitro conditions remain to be explained.

Morphological changes in dimethylbenz(a)anthracene-induced mammary tumours of the rats were accompanied by loss of hormone dependency as well as an increase in growth rate [14]. In the present experiments, growth rate of the spindle-shaped cells was slower than that of the original round cells and it was maintained rather constantly regardless of androgen levels. Since tumour-bearing castrated animals survived longer, death might be induced by proliferation of the round cells. Increase of the spindle-shaped cells seemed to be a less significant influence on the life span of the animals. Moreover, the round cells were able to preserve androgen dependency after growing in castrated mice. It was reported that these cells showed androgen dependency after long passages in male and female athymic nude mice [9]. From these observations, the round cells seem to have a high proliferating potency and adaptability under conditions with various androgen levels. On the contrary, relapse of rat prostatic tumour of R 3327 after castration occurs by an increase of pre-existing androgen-insensitive tumour cells [16]. In the case of the SC 115, pre-existing androgen-insensitive cells, which are assumed to be the spindle-shaped cells, occupy a small part in the tumours before and after castration.

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