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Hormone-dependent Uterine Sarcomas in GR Mice

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Abstract—*Spayed GR mice treated with progesterone and estrone develop mammary tumors within* 3 weeks. *The present paper demonstrates that uterine sarcomas develop in a high percentage of those animals that survive* 17 weeks *of hormone treatment. The growth of the tumors is hormone dependent, and estrogen as well as progesterone receptors were demonstrated in the tumor tissue. Tumor cells were cultivated in monolayer culture. After subcultivation the cells retained their hormone dependence as tested by retransplantation* in vivo. *The uterine tumors in the GR mouse are suggested as a supplementary model to the widely used mammary tumors to investigate steroid hormone action on tumor growth.*

INTRODUCTION

SPAYED GR mice treated with progresterone (P)+estrone(E) develop microscopically detectable mammary tumors within 3 weeks [1]. After 14 weeks of treatment, 90% of the animals bear palpable mammary tumors, most of which are hormone dependent. These tumors have been a valuable tool in the investigation of differences between hormone-dependent and -independent tumors [2–9].

In the same strain of mice we have observed that among those animals that survive 17 weeks of treatment some develop mesenchymal tumors. Since these tumors may provide a model for the study of hormonal regulation of tumors we have investigated the hormone dependence of these tumors as well as their content of estrogen and progesterone receptors. Furthermore, tumor cells were cultivated in monolayer culture.

MATERIALS AND METHODS

Animals

The inbred GRS/AFib strain of mice that carries the mammary tumor virus was used in all experiments. The mice received standard laboratory rat diet (Rostock) and were given both food and water *ad libitum*. They were kept under a constant light regimen of 12/12 hr light/dark in makrolon II cages, 36 × 24 × 15 cm with 15 mice per cage or 26 × 20 × 14 cm with 4–10 mice per cage.

Tumor induction

Eleven-week-old female mice were spayed. Through an incision in each flank the ovary was found and the distal end of the uterus with the ovary was removed. The same day, treatment with P+E (1) was initiated. P was given as three pellets (10 mg) s.c. per week and E was administered in the drinking water in a concentration of 0.5 μg/ml. In about 90% of the animals, mammary tumors arise within 14 weeks of treatment [2].

One hundred and fifty-six animals that had survived 14 weeks of hormone treatment were autopsied 14–25 weeks after the initiation of hormone treatment. The occurrence of histologically verified uterine tumors was recorded.

Transplantation of tumors and tissue cultured cells

Uterine tumors were transplanted s.c. to castrated male mice treated with P+E, P, E, or untreated. Each animal received 50–100 μl minced tumor tissue. Monolayer cultures were treated with 0.25% trypsin to detach the cells. The cell suspension was centrifuged at 150 *g* for 10 min. The cell pellet was resuspended in fresh medium and approximately 2 mg cell protein per inoculation was given s.c. into castrated male mice, some of which were

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treated with P+E. The animals were observed every week for 3 months.

Hormone dependence

A tumor was defined as hormone dependent (HD) when transplantation of the tumor to castrated male mice resulted in tumor growth only in hosts treated with hormones.

Tissue culture

Primary monolayer cultures were obtained by passing minced tumor tissue through a stainless steel grid (mesh size 0.4 mm) and seeding the cell clumps in plastic T-flasks in a medium containing a modified* MEM + 20% fetal calf serum. The medium was renewed three times weekly and at these times the flasks were flushed with a gas mixture of 5% CO_2 + 20% O_2 + 75% N_2. Subcultivation was carried out using 0.25% trypsin (Difco 1.250).

Hormone receptor assay

Estrogen and progesterone receptors were determined by the dextran-coated charcoal method as previously described [10]. Based on Scatchard plot analysis, the receptor binding capacity was expressed as femtomoles hormone specifically bound per mg of cytosol protein.

RESULTS

The incidence of uterine tumors in spayed GR mice treated with P+E for more than 17 weeks was 15/101 (15%, 95% confidence limits: 9–24%). The tumors ranged from 1 to 10 mm in diameter and were located in the upper part of the uterine horn closely related to the site of resection. Only 3/57 (5%) animals developed tumors during treatment for 17 weeks or less. In intact female mice, no uterine tumors were found in 124 mice (95% confidence limits: 0–3%) that were observed until time of spontaneous death.

Microscopically, the tumors consisted of mesenchymal cells that demonstrated varying degrees of nuclear polymorphy (Fig. 1). Few mitoses were observed and the tumor did not demonstrate a convincing invasive growth patter. Upon macroscopic examination no metastases were found in the lungs, the liver, the kidneys, or the spleen. Special stains for muscle tissue (van Gieson) revealed a slight positive yellow color and electron microscopy (Fig. 2) showed that many of the tumor cells contained fine fibrillar material measuring 70–80 Ångstrom, which is characteristic of smooth muscle filaments [12]. However, focal densities, pinocytotic vesicles or basal lamina material characteristic of muscle cells could not be demonstrated. No other sarcomatous tumors nor carcinomas were found.

The tumors could be succesfully transplanted to castrated mice if these were treated with P+E (Table 1). For transplanted tumors, treatment with P alone was usually sufficient to support tumor growth. One tumor line (1347ou) was also transplantable to castrated mice treated with E alone.

Estrogen as well as progesterone receptor protein were demonstrated in the transplanted tumors growing in P+E treated animals as well as in those growing in animals treated with P alone. Neither estrogen nor progesterone receptor could be demonstrated in the tumor growing in an untreated animal (1347ou—6th transplant generation). Receptor content varied considerably between tumors in different transplant generations. In the tumor line 13393u, the mean estrogen and progesterone receptor content were 145 (range 43–249, $n=8$) and 243 (range 99–366, $n=7$) femtomoles per mg cytosol protein with a K_D value ranging between 2 and 13×10^{-10} M for both receptors.

Monolayer cell cultures were established from three tumors (Fig. 3). Characteristically, large granules were observed in many of the cells. The cultures were serially passed through more than 20 passages *in vitro*. Subcutaneous reinoculation of the cultured cells into castrated mice gave rise to hormone-dependent tumors (Table 2) with the same histological appearance (Fig. 4) and hormone receptor pattern as that of the original tumor.

DISCUSSION

Spontaneous uterine tumors are rare in mice [13]. However, sarcomas of the uterus have appeared in 20% of BALB/c mice treated with P for 18 months [14]. One per cent of CF-LP mice also developed uterine sarcomas after about 19 months of treatment with oral contraceptives of various types, all containing gestagens ± estrogens [15]. Treatment of C57 Black mice with testosterone have also induced uterine tumors, probably of decidual origin [16]. In this latter strain of mice no uterine tumors were found after treatment with P+E [17]; however, the treat-

*Minimum essential medium [11] with double concentration of amino acids, four-fold concentration of vitamins and glutamine, and 30% higher concentration of glucose. Penicillin and streptomycin were added at concentrations of 250 IU per ml and 25 μg per ml, respectively.

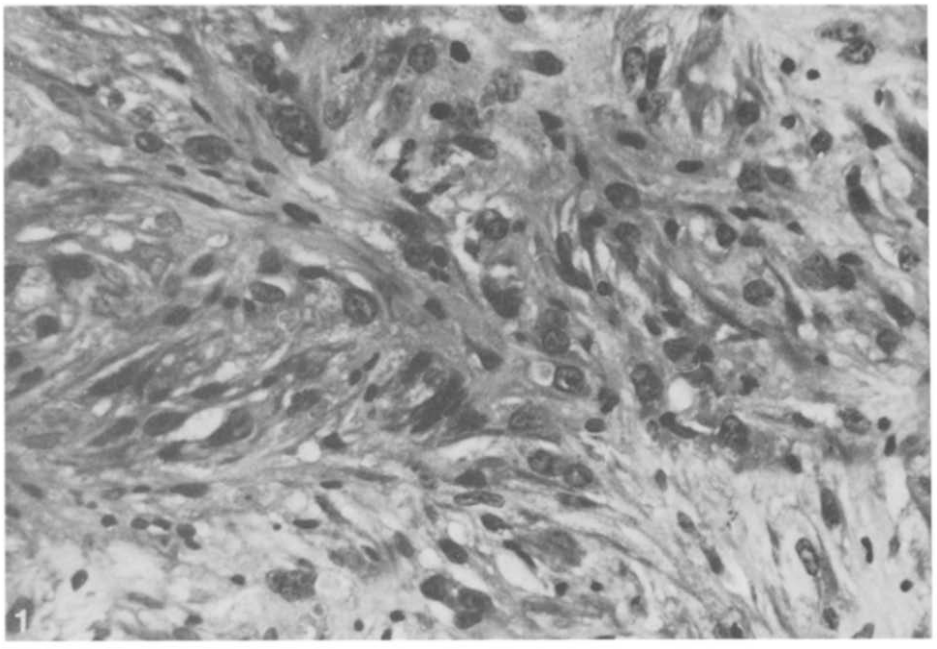

Fig. 1. *Uterine tumor.* H-E × 400.

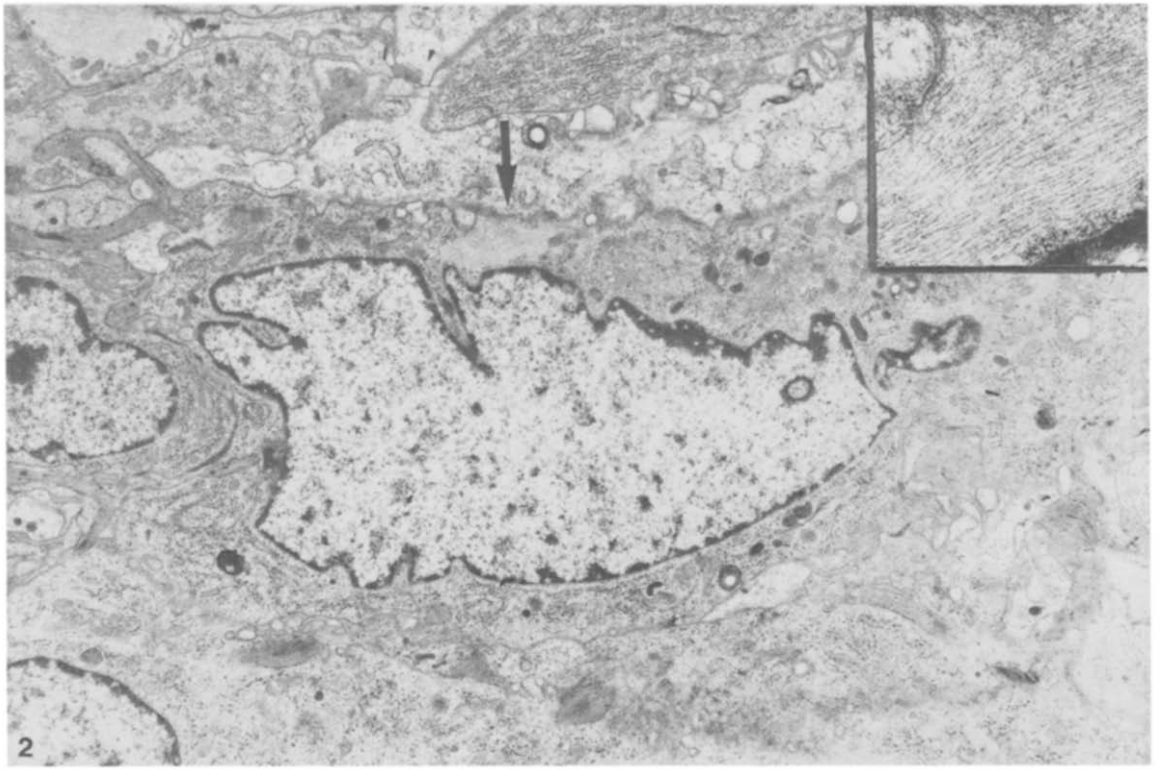

Fig. 2. *Electron micrograph of uterine tumor demonstrating mesenchymal tumor cell of myo-fibroblast type* (× 6800). *Arrow indicates fine fibrillar material also shown as inset* (× 38,000).

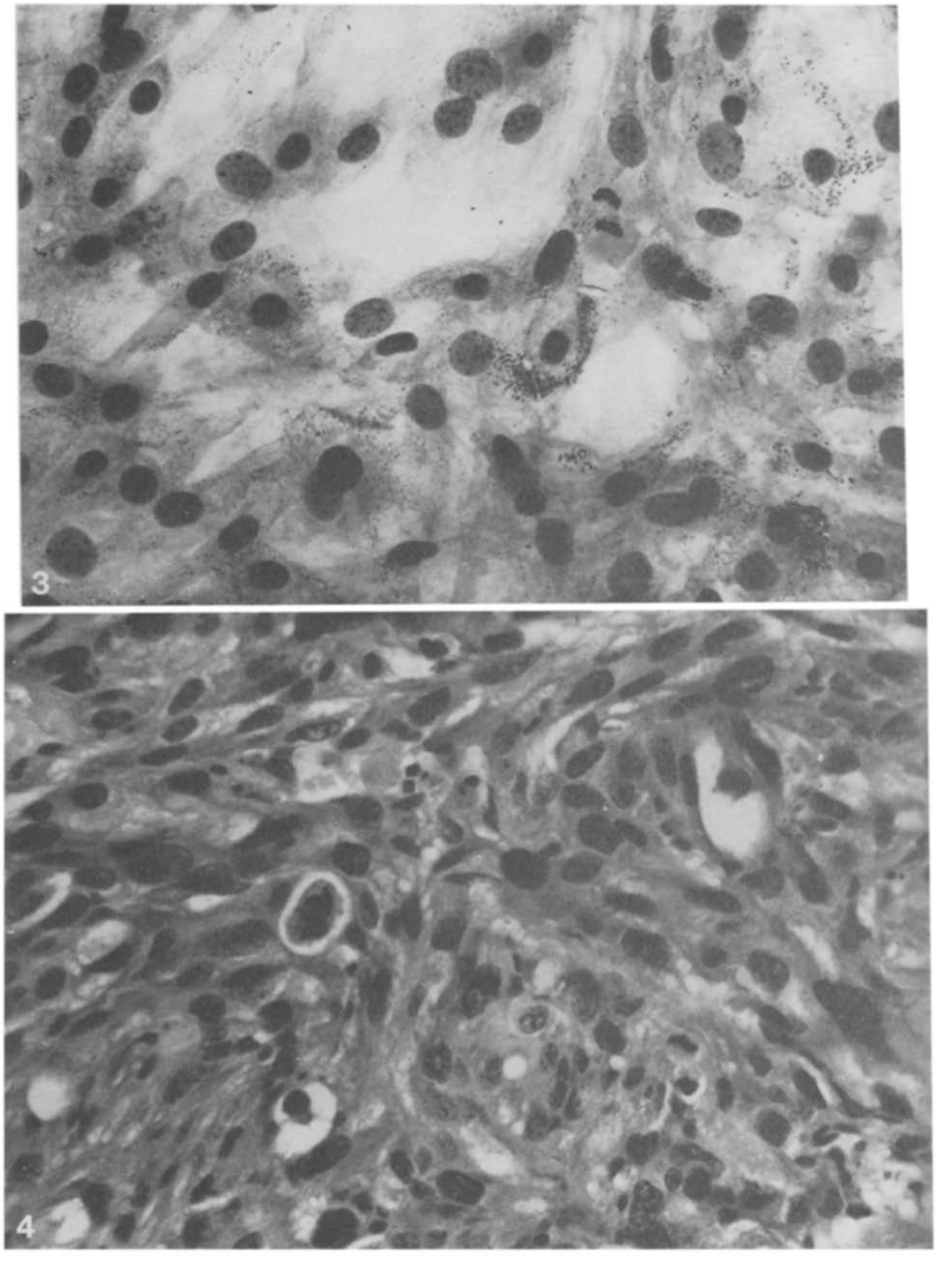

Fig. 3. Tissue culture of uterine tumor. H-E × 214.
Fig. 4. Tumor after inoculation of cultured cells from uterine tumor. H-E × 400.

Table 1. Hormone dependence of serially transplanted uterine tumors

Transplant generation	No. of animals with tumor / No. of animals inoculated									
	1	2	3	4	5	6	7	8	9	10
Tumor 13393u										
P + E*)	2/2	2/2	2/2	2/2	2/2	3/3	2/2	2/2		
P	n.t.‡)	o/2	2/2	2/2	2/2	o/2	2/2	1/2		
E	n.t.	o/2	o/2	o/2	o/2	o/2	o/2	o/2		
-	o/2	o/2	o/2	o/2	o/2	o/2	o/2	o/2		
Tumor 1355ou										
P + E	2/2	1/1	1/2	2/2	1/1	4/4	2/2	2/2	2/2	n.t.
P	n.t.	o/2	n.t.	o/2	n.t.	o/2	2/2	2/2	2/2	2/2
E	o/2	o/2	n.t.	o/2	1/2	o/2	o/2	o/2	o/2	n.t.
-	o/2	o/2	o/2	o/2	n.t.	o/2	o/2	o/2	o/2	o/2
Tumor 16o64u										
P + E	2/2	1/1	3/3	3/3	2/2					
P	n.t.	2/2	1/2	1/2	2/2					
E	n.t.	o/2	o/2	o/2	o/2					
-	o/2	o/2	o/2	o/2	o/2					
Tumor 1347ou										
P + E	2/2	2/2	3/3	2/2	2/2	2/2	1/2	lost		
P	n.t.	2/2	2/2	n.t.	n.t.	2/2	o/2			
E	n.t.	1/2	1/2	n.t.	n.t.	2/2	o/2			
-	o/2	o/2	o/2	n.t.	n.t.	1/2	o/2			

*P: progesterone (10 mg s.c. in pellets weekly).
E: estrone (0.5 μg per ml drinking water).
‡n.t.: not tested.

Table 2. Hormone dependence of uterine sarcomas after growth in monolayer culture

Tumor	Subculture passage	No. of animals with tumor / No. of animals inoculated; Treatment of host: P+E*	Untreated
10215u (from 5. transplant generation)	1	2/2	0/2
	7	1/1	0/2
1347ou (from 2. transplant generation)	1	2/2	0/2
	7	1/2	0/2
	8	1/2	0/2
1355ou (from 1. transplant generation	1	1/2	0/2

*P: progesterone (10 mg s.c. in pellets weekly).
E: estrone (0.5 μg/ml drinking water).

ment period was cyclic and shorter than that of the CF-LP mice. Finally, uterine sarcomas have been induced by chemical carcinogens such as 20-methylcholanthrene. The tumor incidence was decreased by treatment with estrogen and increased by treatment with progesterone or testosterone [18]. The hormone dependence of the uterine tumors was not investigated in any of the above papers.

In the GR strain of mice, uterine tumors have not previously been described. A pleomorphic histology was consistently found, only a few necrotic areas were present and no inflammatory response was noted. The microscopic picture, including electron microscopy, is consistent with a poorly differentiated sarcomatous tumor of myo-fibrous origin. However, invasive growth or metastases have not been found.

Although the uterine tumors appear during hormone treatment it cannot be excluded that

resection of the end of the uterus is important for the tumor development. Trauma has often been suggested as a contributory factor in tumorigenesis and sarcoma development at injection sites in the presence of estrogen have been reported [17, 19, 20].

The uterine tumors in GR mice may be a useful addition to the rare number of animal models of hormone responsive mesenchymal tumors, e.g., rat uterine leiomyoma [21], rat osteogenic sarcoma [22] and rat chondrosarcoma [23]. The uterine tumor model is well suited for the study of steroid hormone action on tumor growth for the following reasons: (1) The tumors maintain their hormone dependence and hormone receptors through serial transplantation, (2) the tumor growth is in most cases supported by P alone and does not always require both P+E as in the case of the hormone dependent mammary tumors, and (3) proliferating monolayer cultures of the tumor cells maintain their hormone dependence *in vivo* after cultivation *in vitro*.

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