Characteristics of an Androgen/Estrogen-induced Uterine Smooth Muscle Cell Tumor of the Syrian Hamster

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SUMMARY

This paper describes the growth and transplantation of an androgen/estrogen-induced, hormone-responsive uterine leiomyosarcoma of the Syrian hamster and of several autonomous variants derived from it. The tumor progresses toward autonomy rapidly. In serial transplantation, short periods (100 to 200 days) of androgen/estrogen treatment favor retention of a typical leiomyosarcoma architectural pattern; long treatment (250 to 350 days) favors progression to a very atypical pattern. Removal of hormone support, following establishment of good transplant growth, favors increased malignancy. Both hormone-responsive primary tumor and autonomous, nonresponsive variants are available in a liquid nitrogen tumor bank.

INTRODUCTION

In 1957 a brief, but illustrated, announcement of an androgen/estrogen-induced smooth muscle tumor of the uterine horns was included in a summary of sex hormone-induced tumors in Syrian hamsters (10). The first (preliminary) report of the tumor was made in 1951 (3). Since then it has been referred to several times without adequate description (4, 5, 12-14, 17, 19). These reports have been confirmed by another laboratory (20-23).

The purpose of this paper is to provide a definitive account of the induction, growth, and transplantation characteristics of the androgen/estrogen-dependent uterine neoplasm; to characterize its morphology; and to describe its acquisition of autonomy.

MATERIALS AND METHODS

The species used in this study was the golden Syrian hamster, Mesocricetus auratus, including a partial albino variety (see references in Ref. 16). The animals were obtained from several California dealers and from our breeding colony; none were from inbred stock. They were maintained in cages with wire bottoms, with 1 to 5 animals in a cage. Untreated, mature females were caged individually, since they are more aggressive than untreated males. All were given water and Purina laboratory chow or Wayne Lab Blox ad libitum with greens added twice a week. Room temperatures were maintained at about 75°F.

Diethylstilbestrol and all steroidal hormones used were, for the most part, implanted subpannicularly as compressed pellets of the pure chemical: 30 mg for the androgen and 20 mg for all other pellets. A few of the animals in early experiments received subpannicular injections of 0.6 mg estrogen suspended in 0.2 ml 0.9% NaCl solution plus 1.0 mg testosterone propionate every other day, usually starting on the 50th day of life. Later, a few animals received the suspension followed by pellet implantation. Both methods of administration were effective. The mean daily absorption was 0.11 mg for diethylstilbestrol and 0.15 mg for testosterone propionate.

Surgical procedures used were essentially the same as those customarily used with rats. For transplantation, healthy tumor tissue was prepared semiquantitatively by forcing it through stainless steel screens of 013-gauge wire, 30 meshes to the inch, into a previously weighed vial containing 1 ml 0.9% NaCl solution, which was then reweighed and diluted to a concentration of 5 mg/ml, and injected subpannicularly in 1-ml aliquots per animal. No screen was used a second time without being sterilized. In the great majority of successive serial passages, only 1 host was used.

Tissues were prepared for storage in a liquid nitrogen bank as follows: 10 mg finely chopped tissue were suspended in 1 ml of the following mixture: Solution 199-IX (Hanks’ base), Grand Island Biological Co., Grand Island, N. Y., 75 ml; fetal calf serum (type 11), Grand Island Biological Co., 15 ml; dimethyl sulfoxide, Baker’s Analyzed Reagent, 10 ml. The suspension was sealed in a 1-ml ampoule (Kimax Ampul.breeder, 12090) and immersed for 30 min in a 1% aqueous solution of méthylène blue. If no dye penetrated into the suspension, the ampoule was stored in a Linde liquid nitrogen refrigerator type LNR-35 with a Linde biological freezer type BF-5, set to lower temperature about 1°/min.

Tissues were fixed routinely in Bouin’s fluid, embedded in paraffin, sectioned at 7 μ, and stained with Harris’ hematoxylin and aqueous eosin or by the Mallory azan procedure. Occasional preparations were stained by other methods, e.g., Foot’s silver ammonium carbonate and phosphotungstic acid-hematoxylin.
RESULTS

The androgen/estrogen-induced uterine tumors were first observed (July 1950) in a 395-day-old hamster which had been treated with subcutaneously implanted compressed pellets of pure diethylstilbestrol and testosterone propionate (20 and 30 mg, respectively) for 345 days. At autopsy a small tumor nodule was observed near the cervical end of the right uterine horn and a considerably larger nodule was seen near the middle of the left horn. Both nodules were diagnosed originally as leiomyomas (3). Similar tumors were observed subsequently in 105 females receiving prolonged (192 to 509 days) androgen/estrogen treatment. Some of these tumors were transplanted into untreated, androgen-treated, estrogen-treated, and androgen/estrogen-treated intact and gonadectomized males and females. Distinct growth occurred only in hosts receiving both testosterone propionate and diethylstilbestrol, although slight growth was supported by the estrogen alone. In several instances, 17β-estradiol was substituted for diethylstilbestrol, both during induction and transplantation; it was equally effective.

None of 14 intact females or 7 ovariectomized hamsters treated with diethylstilbestrol, testosterone propionate, and progesterone for 214 to 400 (mean 268) days acquired uterine tumors.

The mean growth rate and the latent period for induction are unknown. However, of 457-day-old animals treated for 192 days, 1 had multiple large tumors in each horn, 1 had a solitary medium-sized tumor in the right horn, 1 had tiny solitary tumors in each horn, and 1 had a tiny solitary nodule in the left horn. At the other extreme, 2 animals treated for 406 days had only tiny but multiple uterine tumors, one in the left horn, the other in each horn. These observations indicate considerable variation in the latent period required for tumor induction. No metastases from primary lesions have been observed. Removal of androgen/estrogen pellets, following laparotomy confirmation of tumor presence, was followed by rapid tumor regression.

As in males with leiomyosarcomas of the ductus deferens (1, 16, 18) the leiomyosarcoma never appeared to be a direct cause of death. The maximum age at death of a hamster with primary uterine leiomyosarcomas was 717 days. This animal possessed also a poorly differentiated carcinoma of uncertain origin; nevertheless, androgen/estrogen-treatment did tend to shorten the life-span.

The tumors arose in the myometrium (Fig. 1) accompanied by cystic glandular hyperplasia in the uterine mucosa (3). The locus of tumor inception varied and involved each muscle layer. As with the ductus deferens tumor (17), there was, at the outset, focal nodular hyperplasia in the muscle of 1 or both uterine horns. Morphologically, there was no striking alteration in muscle cell morphology in subsequent discrete tumor nodules (Fig. 2), although the individual cells tended to shorten considerably. The interlacing fascicular pattern tended to be preserved. In animals that had been under long-term androgen/estrogen treatment, tumors were usually multiple (Fig. 3) and, in contrast to the ductus deferens leiomyosarcoma (16), followed a random distribution ranging from the proximal to the distal (cervical) ends of the horns (Fig. 4). Multinucleated cells occurred (Fig. 5), but rather sparsely. Rarely, these tumors were found in the cervical region in which case they tended to be larger than those occurring in the horns.

Hemorrhagic necrosis, localized extravasation of blood, or liquefaction were not characteristic of the tumors.

Early transplants of the tumor resembled primary lesions (Fig. 6); however, after prolonged serial transplantation (e.g., 30 passages), some morphological change was occasionally evident. When growth was rapid, the intertwining fascicles became less evident, because the formerly elongated, spindle-shaped tumor cells comprising them became shorter, more blunt, and somewhat epithelioid. This was associated with more abundant mitoses, some of which were abnormal; increased size and number of nucleoli, suggestive of the ductus deferens tumor (16); and an increased frequency of both mononucleate and multinucleate giant cells. Transplants frequently attained weights exceeding those of host animals (Fig. 7).

Primary uterine tumors from 9 different donors were transplanted into androgen/estrogen-treated hosts, as summarized in Table 1. Two were discontinued following the 1st serial passage, 3 after the 2nd passage, and 1 after the 8th passage. The remaining 3 were continued for 11, 17, and 85 passages, respectively, after which they were preserved in the tumor bank, as was 1 primary tumor not yet transplanted in vivo.

In the first of these 3 (No. 7174) the 1st serial passage grew in all of 4 androgen/estrogen-treated females. The 2nd passage grew in both of 2 similarly treated females. On the other hand, it grew equally well in all of 6 untreated females. It failed to grow in the only untreated male used. The 3rd passage grew in 4 of 4 untreated females. Subsequently, it was carried only in untreated female hosts, except for 2 males receiving the 10th passage; again, the transplant failed to grow. During the in vivo history of this tumor, transplant growth occurred in all of 31 female, but in none of 9 male, hosts used. It was discontinued after the 11th and 12th passages were frozen in the tumor bank. The mean latent period for palpability of this transplant decreased from 162 days for 6 androgen/estrogen-treated female hosts to 112 days for 25 untreated females. The actual range, however, was considerable, the shortest period for treated animals being 114 days as contrasted to 41 for untreated animals. In all untreated hosts, showing transplant growth, there was a consistent decrease in latent period from the 1st to the final passage (Table 1). All transplants, in androgen/estrogen-treated as well as in untreated hosts, remained histologically typical leiomyosarcomas resembling the original tumor (Fig. 8).

The first autonomy check on the 2nd of the 3 (No. 9147) was from the 3rd passage and was made in 2 intact males. Unlike No. 7174, growth occurred in both with a mean rate of 330 mg/day and a mean latent period of 27 days. This autonomous line was discontinued after 17
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TABLE I
Serial transplantation history of 9 androgen/estrogen-induced uterine leiomyosarcomas in Syrian hamsters

<table>
<thead>
<tr>
<th>Original donor*</th>
<th>Sex</th>
<th>Mean latent period (days)</th>
<th>Mean transplant growth rate (mg/day)</th>
<th>Takes/sample</th>
<th>Serial passage</th>
<th>Treated or untreated</th>
<th>Sex</th>
<th>Mean latent period (days)</th>
<th>Mean transplant growth rate (mg/day)</th>
<th>Takes/sample</th>
<th>Preserved in tumor bank</th>
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<tr>
<td>6738 S + Tpr</td>
<td>¥</td>
<td>237</td>
<td>55</td>
<td>3/3</td>
<td>1 S + Tpr</td>
<td>¥</td>
<td>237</td>
<td>55</td>
<td>3/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6846 S + Tpr</td>
<td>¥</td>
<td>197</td>
<td>3</td>
<td>2/2</td>
<td>1 S + Tpr</td>
<td>¥</td>
<td>197</td>
<td>3</td>
<td>2/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2235 S + Tpr</td>
<td>¥</td>
<td>Unknown</td>
<td>Small, but viable</td>
<td>1/2</td>
<td>2 S + Tpr</td>
<td>¥</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>2/2</td>
<td></td>
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<tr>
<td>S</td>
<td>¥</td>
<td>Unknown</td>
<td>0.2</td>
<td>1/1</td>
<td>2 S</td>
<td>¥</td>
<td>~120</td>
<td>2</td>
<td>3/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tpr</td>
<td>¥</td>
<td>No growth</td>
<td>No growth</td>
<td>0/1</td>
<td>2 S</td>
<td>¥</td>
<td>~120</td>
<td>2</td>
<td>3/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>¥</td>
<td>No growth</td>
<td>Tiny, but viable</td>
<td>0/1</td>
<td>2 S</td>
<td>¥</td>
<td>~120</td>
<td>2</td>
<td>3/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2329 S + Tpr</td>
<td>¥</td>
<td>&lt;100</td>
<td>109</td>
<td>3/4</td>
<td>2 S</td>
<td>¥</td>
<td>&lt;120</td>
<td>Small, but viable</td>
<td>5/5</td>
<td></td>
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<tr>
<td>S + Tpr</td>
<td>¥</td>
<td>&lt;100</td>
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<td>3/4</td>
<td>2 Tpr</td>
<td>¥</td>
<td>Unknown</td>
<td>No growth</td>
<td>0/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S + Tpr</td>
<td>¥</td>
<td>&lt;100</td>
<td>109</td>
<td>3/4</td>
<td>2 U</td>
<td>¥</td>
<td>Unknown</td>
<td>Small, but viable</td>
<td>1/2</td>
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<tr>
<td>6541 S + Tpr</td>
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<td>42</td>
<td>3/3</td>
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<td>¥</td>
<td>&lt;28</td>
<td>114</td>
<td>2/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S + Tpr</td>
<td>¥</td>
<td>&lt;170</td>
<td>115</td>
<td>3/3</td>
<td>5 S + Tpr</td>
<td>¥</td>
<td>&lt;28</td>
<td>114</td>
<td>2/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7174 S + Tpr</td>
<td>¥</td>
<td>130</td>
<td>~180</td>
<td>4/4</td>
<td>11 U</td>
<td>¥</td>
<td>52</td>
<td>68</td>
<td>2/2</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>9147 S + Tpr</td>
<td>¥</td>
<td>76</td>
<td>263</td>
<td>2/2</td>
<td>17 U</td>
<td>¥</td>
<td>28</td>
<td>2364</td>
<td>1/1</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>S + Tpr</td>
<td>¥</td>
<td>76</td>
<td>263</td>
<td>2/2</td>
<td>17 U</td>
<td>¥</td>
<td>28</td>
<td>10</td>
<td>1/1</td>
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<td>-</td>
</tr>
<tr>
<td>6580 S + Tpr</td>
<td>¥</td>
<td>134</td>
<td>303</td>
<td>1/1</td>
<td>90 U</td>
<td>¥</td>
<td>6</td>
<td>5775</td>
<td>1/1</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

* Primary tumor, 13482Q, preserved in tumor bank, but not transplanted in vivo.

1, diethylstilbestrol; Tpr, testosterone propionate; U, untreated.

Calculated based on 3 of 4 animals. Fourth animal treated with S + Tpr for only 200 days, followed by 470 days without treatment. Transplant became palpable 297 days after cessation of S + Tpr treatment; had growth rate of only 1 mg/day over entire 670 days, but weighed 938 mg at autopsy.

passages. In all autonomous passages, the characteristic histology of the original leiomyosarcoma was retained.

The 5th serial passage of the 3rd of these 3 (No. 6580), in androgen/estrogen-treated hosts, was the source of 2 separate autonomous lines; one was the result of transplanting directly into untreated hosts, and the other was derived from a transplant in a host given androgen/estrogen treatment for 353 days followed by 209 days without treatment: Withdrawal of treatment was followed by an initial decrease in transplant size succeeded by a renewal of growth. In a 3rd line from the same source, transplants were continued in androgen/estrogen-treated hosts. Data relative to specific tumor weights after a given period of time and growth rates for individual transplants cannot be presented in a very meaningful way, since survival times after transplantation for different hosts were variable; nevertheless, the general trends were unmistakable. Study of these data and of histological preparations support these 2 conclusions:

Continued serial transplantation into androgen/estrogen-treated hosts favored a gradual decrease in latent periods and a corresponding increase in the rate of transplant growth. Short periods of treatment, e.g., 100 to 200 days, favored retention of a typical leiomyosarcoma architectural pattern; relatively long periods of treatment, e.g., 250 to 350 days, favored progression to a very atypical pattern characterized by marked anaplasia and giant cell formation.

Removal of hormone support, following the establishment of good transplant growth, resulted in an initial decrease in transplant size, succeeded by a renewal of growth, accompanied by acquisition of the above atypical pattern (Fig. 9). Serial transplants from this source retained the pattern and exhibited an immediate, instead of gradual, decrease in latent periods for palpability and a corresponding increase in growth rates.

This 2nd autonomous line grew equally well in intact and gonadectomized male and female hosts. It remained constant during 85 serial passages after which it was preserved in the tumor bank and discontinued in vivo. During the 85 passages, its mean latent period for palpability was 6 days, and its mean daily growth rate was 3222 mg.
DISCUSSION

Since early reports of Gardner (8) and others, it has been generally understood that in hormone-induced, hormone-dependent tumors it is primarily cell proliferation rather than viability that is dependent. Such an induced tumor, when transplanted into an intact host, preferably gonadectomized, may be hormone responsive, but may regress without such hormonal support. Later, if such responsiveness disappears and the transplant grows without hormones, it is said to be autonomous-nonresponsive (7). The growth rate of some tumors can be increased or decreased, but not prevented, by endocrine manipulation; such tumors have been considered autonomous yet hormone responsive (7). Huseby (9) and others have suggested using the presence or absence of growth in hypophysectomized hosts as a criterion of dependency or autonomy. It is obvious that 2 different phenomena, proliferation and viability, are intimately involved in tumor dependency and autonomy. This applies to whole tumors rather than to their constituent cells, since data are not available concerning cell survival and generation time. Cell proliferation could conceivably be appreciable in a regressing tumor if cell loss rate were high.

In the leiomyosarcoma considered in this paper, and in many other “responsive” tumors as well, it is proliferation, not viability, that is hormone dependent. Although generally recognized, this distinction frequently receives less emphasis than it deserves, e.g., in a recent paper (6) an estrone-induced rat mammary gland carcinoma is described as showing prompt and “complete regression” in the absence of estrone. Since the tumor became rapidly reestablished following estrone treatment, it is apparent that it was ability to proliferate, not viability, which was dependent. Regression of such tumors cannot properly be referred to as “complete.” A sharp distinction, therefore, should be made between proliferation and viability in any discussion of tumor dependency.

The addition of progesterone to androgen/estrogen completely suppressed tumor induction in intact (maximum duration of treatment, 287 days) and gonadectomized (maximum duration of treatment, 400 days) females. However, in 2 of a series of males treated with progesterone plus androgen/estrogen, tiny tumors were evident in the epididymal ends of the ductuli deferentia after 440 and 503 days, respectively (unpublished data). This suggests that longer treatment of females might have induced tumors. Somewhat larger tumors were present after 386 days of cortisone/androgen/estrogen treatment in 1 of a series of males (unpublished data); this treatment was not repeated with females.

The common neoplastic response of the musculature of the uterine horns and ductuli deferentia to treatment with combined male and female sex hormones in the hamster is not understood. If it results from a common origin of the Wolffian and Mullerian ducts from the urogenital ridge, muscle of the oviducts, vagina, seminal vesicles, prostate, and epididymal heads would be expected to participate; this does not occur. That phylogenetic and ontogenetic changes in hormone sensitivities of the genitourinary system are retained, to varying degrees, in the adult hamster is indicated, in the field of experimental neoplasia, by the estrogen-induced renal carcinoma (11), the androgen/estrogen-induced uterine horn and ductus deferens leiomyosarcomas (16) and estrogen-induced epididymal head hyperplasias (unpublished data).

In view of the positive nature of lactic dehydrogenase, isoenzyme studies (2) made on the androgen/estrogen-induced flank organ (scent gland) tumor of the hamster which, as has been emphasized (15), has an extremely leisurely histogenetic history, it might be profitable to conduct similar studies on the uterine horn tumors, especially during the critical period of their conversion to autonomy.

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