Characteristics of an Androgen / Estrogen-induced, Dependent Leiomyosarcoma of the Ductus Deferens of the Syrian Hamster

I. *In Vivo*.[1]

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SUMMARY

Hormone-dependent leiomyosarcomas of the ductus deferens/epididymal tail complex appeared in virtually 100% of Syrian hamsters treated simultaneously with androgen and estrogen for approximately 400 days. Hypophysectomy did not prevent tumor induction. These tumors showed no change in hormone dependency after 60 serial passages. Neither hormone alone supported growth. Primary tumors and early transplants had a strongly fasciculated architecture and nucleoli were inconspicuous. By the sixth transfer, very prominent nucleoli became characteristic of the tumor cells and there occurred a rearrangement of fascicles into cords and columns. Concomitantly the latent period decreased from 200 to 40 days, approximately.

We know of only three neoplasms inducible by means of prolonged simultaneous administration of both male and female sex hormones (7,9,10). This contrasts sharply with the abundance of tumors which appears following estrogen treatment (6) and with the paucity of tumors associated with androgen treatment (8). The apparent complicity of these two hormones in tumorigenesis is incongruous with the well known antagonism between them in hormone tumorigenesis in general (11, 12).

The first paper of this series (9) described the biology of an androgen estrogen-induced hair follicle epithelioma in the flank organ (scent gland) of the Syrian hamster. The present paper is concerned with one of the two remaining androgen/estrogen inducible tumors, a smooth muscle tumor originating in the muscularis of the epididymal tail/ductus deferens complex.

The androgen/estrogen-induced tumor of smooth muscle (leiomyosarcoma) was first observed on February 28, 1950, in the epididymal tails of a 320-day-old hamster treated for 270 days with testosterone propionate and diethylstilbestrol. On July 5th of the same year the third neoplasm was observed in the uterine horns of a 395-day-old animal treated for 345 days. Histologically it resembled the ductus deferens tumor. A brief preliminary illustrated account of the tumors is already available (7). Since then the smooth muscle tumors have been induced in 227 males and 98 females. The ductus deferens tumor has been transplanted successfully as a hormone-dependent neoplasm from twelve male donors; one of these is now in the 60th serial transfer.

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MATERIALS AND METHODS

Materials and technics employed were similar to those already described (9).

OBSERVATIONS

The simultaneous administration of exogenous androgen and estrogen, in the form of testosterone propionate (or base) and estradiol (or the synthetic estrogen diethylstilbestrol)—either by injection of microcrystalline suspensions, sesame oil solutions, subpannicular implantation of pure pellets, or even topical application to the skin—resulted in a very gradual multifocal hyperplastic reaction in the distal portion of the epididymal muscularis (Fig. 2). Subsequently, distinct nodules arose not only in the epididymal tail but in the wall of the adjacent ductus deferens. Ultimately, these neoplastic nodules often appeared along the entire course of the ductus deferens. Usually this condition was bilateral and as much as 39 gm of tumor tissue was recovered from one animal, more than one-half the remaining body weight (Fig. 1).

Although there was some variability, treatment for 300 days usually produced some tumors and, after an additional 100 days, tumors had been induced in virtually

100% of the treated animals. From subpannicularly implanted pellets the mean daily absorptions were 0.11 mg for diethylstilbestrol and 0.15 mg for testosterone propionate.

Attempts to induce tumors by substitution of other estrogen inhibitors or antagonists, e.g., progesterone, deoxycorticosterone acetate, were uniformly unsuccessful, although their inclusion did not prevent tumor induction by androgen and estrogen.

The administration of estrogen alone was followed by marked gross involution of the male sex accessories including the epididymal tail ductus deferens complex. Exogenous androgen, on the other hand, stimulated these target tissues but, even in doses ten-fold those used in combination with estrogen to produce tumors, it completely failed as a tumorigenic agent.

If after an induction period of 200–300 days, androgen/estrogen treatment was interrupted, the hyperplasia and neoplastic response ceased and regression occurred (Figs. 4, 5). Animals autopsied 100 days following cessation of treatment always had apparently normal sex tracts. When animals with large palpable tumors were killed 200 days after cessation of treatment, their epididymal tail/ductus deferens complexes were essentially normal, except for occasional fluid-filled cysts, purulent areas and scarring which marked the tumor regression sites.

When one or the other inducing hormones was withdrawn following a minimum period of 400 days—i.e., long enough to secure a 100% induction—the remaining hormone, either androgen or estrogen, not only prevented complete regression but actually maintained tumor cells in very good condition for at least 200 days, although no further growth occurred. Subsequent transplantations of such static tumor tissue into androgen estrogen-treated hosts resulted in renewed tumor growth.

A line of serial transplants from one of the initial primary inductions is now in the 60th generation. Autonomy checks, i.e., transplantation to nonhormone treated or castrated hosts have been uniformly negative throughout its history, inasmuch as palpable growth failed to occur; even a first generation transplant, however, in an untreated host for 200 days became palpable after 200 days of androgen/estrogen treatment, eventually attaining a weight of 1896 mg after 300 days.

The capacity of this "dependent" leiomyosarcoma to survive without apparent growth during long periods of hormone deprivation was not limited to primary tumors but was observed in subsequent transplant generations even after 580 days of deprivation. It appeared however, that survival in a nontreated animal was nevertheless limited, since no recovery of tumor tissue following the introduction of exogenous hormones after 735 days has been obtained.

Since the role of the pituitary gland is always suspect in instances of endocrine carcinogenesis, a small group of animals was hypophysectomized and treated with testosterone propionate and diethylstilbestrol at 100 days of age. Four of seven completely hypophysectomized animals, as judged by thyroid involution and absence of microscopically recognizable pituitary gland remnants, developed small, typical multiple tumors after a mean of 296 (range 167–374) days of treatment.

Histologically, the primary lesions possessed many of the characteristics of the tissue of origin. Architecturally, the tumor tissue consisted of complex, interlacing fascicles; cytologically, myofibrils were conspicuous and resembled those in control material. The primary tumors were at first not well circumscribed but tended to appear in beds of hyperplastic muscle. As the tumor nodules increased in size they tended to acquire some degree of isolation from surrounding tissue but never became obviously encapsulated.

Transplants made from primary tumors retained the early histologic picture but subsequent transplant generations tended to show some modifications. The strongly fasciculated architecture gave way to a more compact cellular structure, at times suggesting cords or columns rather than fascicles. Myofibrils became less obvious, and exceedingly prominent pyroninophilic nucleoli became abundant. In late transplant generations the nucleoli frequently were coarse, irregular in shape, and often multiple (Fig. 3).

Metastases resembled both histologically and behavioristically, the tumors from which they originated.

The latent period for palpation of the first two generation transplants was approximately 200 days. By the sixth transfer this latent period had dropped to 50 days. Subsequent passages, through the current 60th one, have had latent periods of approximately 40 days. Assessment of our histologic samples suggested that the decrease in latency was concomitant with the loss of organization and the acquisition of the prominent nucleoli.

DISCUSSION

Although the biology of leiomyosarcoma appears superficially to be uncomplicated, certain fundamental questions require clarification. If, as appears to be true, smooth muscle hyperplasia is an essential prelude to tumorigenesis, why does androgen alone, in amounts sufficient to cause gross hypertrophy of the muscularis, fail to evoke a tumor response? Estrogen alone is said (5) to evoke a strictly limited hyperplasia of the fibromuscular components of the epididymal tail. In our experience this has never progressed to neoplasia. At present we cannot attribute specific carcinogenicity to either hormone, but must accept the rather vague notion that it is the "push-pull" effect of combined male and female sex hormones which is carcinogenic. If specificity of action must be invoked, it would seem that estrogen may act carcinogenically on smooth muscle maintained in a hyperplastic state by androgen.

Regardless of the mode of hormone action, once a tumor is induced, it can be maintained without growth by either hormone. Withdrawal of both hormones results in rapid and complete tumor involution. Contrasting sharply with this observation is the fact that suspensions of tumor cells introduced subpannicularly into nontreated hosts (or even into castrates), may sometimes lie quiescently as long as 500 days before producing a tumor upon subsequent androgen/estrogen stimulation.

It is possible that a partial, tentative explanation for this difference in response to hormone deprivation can be derived from a consideration of the behavior of the tumor *in vitro*. When primary tumor, or early generation trans-

plant tissue, is dispersed by pretreatment with collagenase or trypsin and subsequently grown in hormone-free tissue culture, individual cells and small aggregates quickly establish a widely distributed outgrowth in which numerous mitoses occur (3, 4). Such preparations may be readily subcultured. Despite this capacity to survive and even grow in a medium containing no sex hormones, these cultured cells when re-introduced into animal hosts grow only in those animals receiving adequate exogenous hormone treatment. Even after such tissue is recycled between culture and host 3 times, no indication of autonomy can be observed.

On the other hand, the same tissue when cultured organotypically dies rapidly unless the medium contains adequate levels of incorporated sex hormones (3, 4).

When fresh tumor tissue is partially dispersed by forcing it through a fine mesh, stainless steel gauze, and then injected as a saline suspension into hosts left without hormone treatment for long periods of time, no transplant growth is discernible. Such dormant tissue may be mitotically activated, however, by the institution of androgen/estrogen treatment. Once an actual tumor, i.e., tumor cells constituting part of an organized histologic pattern, has developed, hormone deprivation results in rapid, complete involution as observed in organotypic cultures. If, as suggested elsewhere (1–4), "dependency" is a tissue-resident phenomenon, involving architectural integrity, it becomes possible to interpret the above observations in terms of degree of dispersion of the transplanted tumor cells.

Maintenance of the hormone-dependent leiomyosarcoma by either hormone alone is difficult to comprehend, especially if an attempt is made to assign "promoting" values to androgen, and "initiating" properties to estrogen. Preliminary assessment of each hormone independently in organotypic culture (unpublished data) confirms the findings *in vivo*, but sheds no further light on possible mechanisms of action.

For some years this tumor was classified as a leiomyoma in spite of histologic suggestions of malignancy. Recently a mesenterial metastasis from a primary lesion has been observed in an old animal bearing huge bilateral tumors. Since it is probable that metastases would be frequent in such long-term treated animals, we now consider the tumor to be malignant.

As in all carcinogenic situations involving endocrine organs or their targets, the problem of direct, versus indirect, action must be raised. Although no decision can yet be made concerning tumor induction, evidence from experiments *in vitro*, and to a lesser extent *in vivo*, favors the view that hormone "dependency" is a tissue-resident phenomenon and that, in this instance, hormones act directly at this level.

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FIG. 1. 572-day-old hamster. Ductuli deferentia implanted subpannicularly with pellets of stilbestrol and testosterone propionate for 522 days. Each duct has been turned caudolaterally, and bears huge multiple tumors weighing 17.2 gm on the left and 21.6 gm on the right (remaining body weight, 108 gm). × 1.5.

FIG. 2. 450-day-old hamster. Section through part of the epididymal tail implanted subpannicularly with pellets of stilbestrol and testosterone propionate for 400 days. Areas within the muscularis show varying degrees of hyperplasia and neoplasia. × 32.

FIG. 3. 29th serial subpannicular transplant of an induced ductus deferens leiomyosarcoma. Host carried subpannicularly implanted pellets of stilbestrol and testosterone propionate from the 58th through the 93rd day of life. Tumor cells are for the most part short, robust spindles and nuclei contain conspicuous nucleoli. × 650.

FIG. 4. 29th serial subpannicular transplant of an induced ductus deferens leiomyosarcoma. Host carried transplant and subpannicularly implanted pellets of stilbestrol and testosterone propionate from the 58th through the 93rd day of life (cf. Fig. 5). × 65.

FIG. 5. 57th serial subpannicular transplant of an induced ductus deferens leiomyosarcoma. Stilbestrol and testosterone propionate pellets implanted subpannicularly from the 64th through the 150th day of life; no pellets were present for final 50 days of life. Although something of the original tumor architecture is discernible, no living tumor cells remain (cf. Fig. 4). × 65.