Development of Androgen-stimulated Transplants of Nb Rat Carcinoma of the Dorsal Prostate and Their Response to Sex Hormones and Tamoxifen

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ABSTRACT

Androgen-stimulated tumors of the dorsal prostate were derived from an estrogen-dependent tumor of Nb rats (Nb-2Pr-E). The change followed repeated androgen treatments in a rat bearing a tumor which had regressed after estrogen removal. The eventual tumor regrowth provided tumor transplant lines, Nb-2Pr-A, which grew in normal or androgenized males, slowly in castrates, but not in estrogenized females. The Nb-2Pr-A growing tumors regressed after treatment with estrogen, and tumors which eventually regrew were autonomous. Slow tumor growth in castrated animals also responded to estrogen treatment. Tamoxifen, an antiestrogen, caused tumors growing in males to become stationary or regress. Regrowth of tumors after they had responded to tamoxifen again responded to castration or estrogen. Tumors which escaped from estrogen treatment or were autonomous did not respond to tamoxifen. The most effective sequence for treatment was tamoxifen followed by castration and, finally, estrogen. Tamoxifen treatment or castration allowed tumors to remain hormone responsive, whereas initial estrogen treatment prevented response to subsequent therapy and led to an autonomous change.

INTRODUCTION

The preceding paper has described the production of adenocarcinoma of the dorsal prostate in Nb rats. It also included the response to hormones of transplanted lines established from an estrogen-dependent adenocarcinoma, Nb-2Pr-E (14). Androgen-stimulated sublines (Nb-2Pr-A) have recently been developed from a tumor which was originally estrogen dependent and would not grow in males (11, 13). This paper will describe a method for the production of androgen-stimulated tumor lines and the response of their transplants to sex hormones and treatment with tamoxifen. Androgen-stimulated tumors grow rapidly in males, less so in castrates, and very slowly in estrogenized females. Since this model appears to mimic closely the disease in humans, courses of treatment have been designed to test initially the effects of a primary treatment and then, when tumor growth recurred, to try other secondary types of therapy on the same animal. A number of carcinomas of the prostate in Nb rats were sent to Dr. J. Drago. His group have confirmed the action of hormones on these tumors not only in Nb rats but also in athymic (nude) mice (2, 6). The autonomous and androgen-stimulated tumor lines have been used for extensive chemotherapeutic studies, and detailed histopathology of the tumors has been reported (3–5, 7).

MATERIALS AND METHODS

The procedures used in this study have been the same as those described in the preceding paper (14). The androgen-sensitive tumor lines, however, were maintained by transplanting the most rapidly growing tumors from groups of TPP3-treated or normal males. TPP pellets were replaced in all rats every 8 to 12 weeks because of their greater solubility. The antiestrogen tamoxifen (base; kindly supplied by Dr. W. Baumgarten, ICI Americas, Inc.) was administered as a weekly s.c. injection of 5 or 1 mg/week in 0.5 or 0.1 ml oil. Tumors were considered to be autonomous if they grew as rapidly in EP females as in males. Tumor size was measured weekly, and the maximum length plus width (cm) was summated.

Steroids were obtained from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Production of Androgen-stimulated Nb-2Pr-A Tumor Sublines. It has been shown that estrogen-dependent transplants of the Nb-2Pr-E adenocarcinoma growing in estrogenized male rats regressed after removal of the stimulating hormone (14). Following protracted but varied periods of regression, spontaneous growth at the tumor site took place. The resulting tumors, when transplanted, were found to have progressed to become autonomous, growing equally well in estrogenized or normal males. Chart 1 shows the regression following EP removal, as described for Nb-2Pr-E tumors. In the 3 rats of Group A, the EP was removed and replaced by TPP at the same operation. In the other 2 animals in Group B, treatment with TPP was started only after regression had taken place for 8 to 9 weeks following EP removal. TPP were replaced at 8- to 12-week intervals in both groups of rats. Tumors in which regrowth ultimately was reestablished were transplanted into estrogenized and androgenized hosts.

It may be seen that regression of the transplanted tumors, which followed EP removal, continued for some weeks in both groups of rats despite repeated treatment with TPP. All tumors, however, eventually showed regrowth commencing after 13 to 40 weeks and were then transplanted. The transplanted tumors which originally would grow only in EP rats now grew in either estrogenized females or normal males. In some cases, however, the growth rate was slower in estrogenized animals than in normals. Transplants of the tumor of the rat shown in Chart 1, Group B, which had regressed for 42 weeks, were maintained in TPP males. After 3 further transplant generations, it

1 The research work was made possible by personal research funds.
2 Senior Research Investigator, Cancer Control Agency, Vancouver. Received October 2, 1979; accepted July 7, 1980.
3 The abbreviations used are: TPP, 10-mg pellet of 90% testosterone propionate plus 10% cholesterol (2 to 3 pellets implanted for each treatment); EP, 10-mg pellet of 90% estrogen + 10% cholesterol.
had developed a preferential growth in males. This tumor, designated Nb-2Pr-A, is described in the subsequent experiments. Transplant lines of the other 4 tumors shown in Chart 1 unfortunately had to be discontinued before it could be determined if androgen-sensitive lines could also be established.

Growth of Transplants of the Androgen-stimulated Tumor Nb-2Pr-A under Varied Hormone Conditions. The growth of transplants from 2 sublines which were developed from the androgen-stimulated tumor in hosts after varied treatments is shown in Chart 2. The observations extended over transplant generations 8 to 35.

The hormonal status of the host may be seen to have markedly affected the growth of tumor transplants. Rapid growth occurred in males although the initial growth was even more rapid in androgen-treated animals. In 3 rats which were castrated at 2 months of age (6 to 8 months previously), the growth of transplants started later and continued more slowly than in normal males. Tumor growth did not occur in 75% of estrogenized females, although a delayed slow growth took place in 30% of estroganized males. The most striking contrast in the growth of transplanted tumors was between androgenized males and estrogenized females.

Regression of Nb-2Pr-A Transplants in Males after Estrogen Treatment, Their Subsequent Growth Escape, and Effects of Secondary Treatments. Transplants of Nb-2Pr-A in 12 male rats were allowed to commence growth, and then the rats received an EP as the primary form of treatment. Complete regression followed in 5 rats and partial regression was seen in 7 rats. (Three rats of the latter group also carried TPP.) Eventually, regrowth of all tumors took place despite the presence of the EP. Secondary types of treatment were then administered (Chart 3).

It may be seen that tumor growth in all rats ceased abruptly after treatment with an EP. Tumor growth was resumed, however, after 4 to 5 weeks of partial regression in the 7 animals shown in Group A. The subsequent growth of the transplant was not affected by removal of the EP in one rat of this group or by the administration of tamoxifen in another. Complete regression followed EP treatment in 5 rats shown in Group B. Tumor growth in these resumed later, but only after 7 to 14 weeks of complete regression. Secondary treatment included castration, removal of the EP in 3 rats, or injections of tamoxifen in 2 rats and was not effective in altering subsequent tumor growth. It was apparent that estrogen inhibited the initial tumor growth, but when subsequent regrowth took place it could not be influenced by further hormone manipulation. Growth of an autonomous subline was not affected by estrogen treatment (not included in Chart 3).

Regression of Nb-2Pr-A Transplants in Castrates after Estrogen Treatment. Tumors transplanted to previously castrated animals grew slowly after a protracted latent period. The effect of treatment of 2 castrated rats with estrogen is shown in Chart 4. The rats were subsequently treated with tamoxifen.

Tumor growth in 2 castrated rats may be seen to be at approximately one-half the rate found in normal males. Once measurable tumor growth was established, both castrated rats received an EP. In both cases, the tumors stopped growing and partially or totally regressed. When tumor growth escaped and was resumed in one rat, despite the EP, tamoxifen treatment caused only a brief cessation of growth. The other animal was treated with tamoxifen while the tumor was in a regressed state. This was shortly followed by the start of tumor regrowth, possibly due to an antiestrogen effect of tamoxifen. It was apparent that when tumor regrowth occurred in the castrate it would still respond to estrogen and probably to tamoxifen treatment.

Effect of Tamoxifen on the Growth of Nb-2Pr-A Transplants. Tamoxifen was injected weekly for 4 weeks into groups of 4 male and 4 female rats, and the effects were noted for the following 4 weeks. In 2 other males, the antiestrogen was given repeatedly at weekly intervals until tumor growth recommenced. One of the rats then received an EP, and the other was castrated. The effects of tamoxifen on the growth of 2 different autonomous lines is also included in Chart 5.

It may be seen that the growth of the Nb-2Pr-A in all treated rats was reduced when compared with a control (Group D). Tumor regrowth did not occur in 4 weeks following 4 weekly injections of the drug (Groups B and C). In 2 animals (Group E), continuing weekly doses led to regression of the growing tumors, and a reduction in the dose from 5 to 1 mg was
which were stimulated by androgen. It was suggested previ-
some animals after estrogenation, but successive transplants
take place, cells were altered; and although growing in females
estrogen was prevented by repeated treatments with testoster-
ostrogenized rats (14). The autonomous change expected to
male rats. Regrowth of regressed transplants was always au-
tumor growing as readily in normal males as in
estrogen was unexpectedly stimulated by estrogen when transplanted 3
weeks later. On the other hand, castration was followed by
tumor regression in the other animal. It may be noted in contrast
that the growth of 2 different autonomous tumors was not
influenced by tamoxifen treatment (Group A).

DISCUSSION

Androgen-stimulated transplant tumor lines were developed
from the estrogen-dependent tumor Nb-2Pr-E. As previously
uescribed, the latter was totally dependent on estrogen for
growth, and growing tumors always totally regressed after
removal of the estrogen. The tumor did not grow in normal
male rats. Regrowth of regressed transplants was always au-
onomous, the tumor growing as readily in normal males as in
estrogenized rats (14). The autonomous change expected to
occur during the period of regression following removal of
estrogen was prevented by repeated treatments with testoster-
one during the regression period. Although tumor regrowth did
take place, cells were altered; and although growing in females
and males, tumors were stimulated by androgen to grow more
rapidly. The first transplant generations would grow slowly in
some animals after estrogenation, but successive transplants in normal or androgenized males apparently selected cells
which were stimulated by androgen. It was suggested previ-
ously that progression in the tumor cells could be directed from
estrogen to androgen dependency for growth (11). It now
seems more likely that a selection of tumor cells stimulated by
androgen was largely responsible for the emergence of andro-
gen-stimulated tumors. Androgen sensitivity of the Nb tumor
lines has been shown by Drago et al. (2) to be a property of the
rat tumor cell which was retained when transplanted to athymic
(nude) mice.

The Nb-2Pr prostatic carcinoma has offered a new type of
model for studies on the influence of hormones on the growth
of both estrogen-dependent and androgen-stimulated tumor
lines. Whereas the former tumor lines required estrogen for
growth, regressed if estrogen was withdrawn, and then eventu-
ally resumed autonomous growth (1, 12), the latter in contrast
were stimulated to grow by androgen (growing slowly in cas-
trated rats), growth was inhibited by estrogen, and regressed
tumors which eventually grew despite estrogen became auton-
omous. The finding that the same primary tumor may
be followed by either estrogen or estrogen-plus-androgen
stimulation. Transplanted tumors of the breast arising from a
dual hormone stimulation, like the prostate, may be developed
as estrogen- or androgen-dependent sublines (10).

The response of estrogen-dependent prostate tumors to
treatment with the antiestrogen tamoxifen was not unexpected,
since the growths of all estrogen-dependent tumors occurring
in Nb rats respond similarly to this drug (14). The finding that
tamoxifen could also check the growth of androgen-stimulated
tumors was unexpected. It was found to be effective, however,
only when given as the primary form of treatment. Tumors
which had escaped the regression caused by estrogen were
not affected by tamoxifen. On the other hand, tumors which
escaped from tamoxifen treatment still responded to castration
or to estrogen. Tamoxifen has not been reported to have
antiandrogen activity, although it has been found in mice to
exert a weak estrogen effect (9). It seems unlikely that such an
action could explain its effect on Nb-2Pr-A tumors. Tamoxifen
in the doses used showed no suggestion of estrogenic action in male Nb rats. Furthermore, tumors which had regressed in rats after treatment with the drug did not show the same response to subsequent treatment as was found in regressed tumors which followed estrogen treatment. It is possible that the response of androgen-stimulated tumor cells to tamoxifen was related in some way to the origin of the primary tumor which yielded cells responding to either estrogen or androgen. If the counterpart of such tumors occurs in humans, the most effective sequence of hormone treatment would be to use tamoxifen as the initial drug, followed by castration and finally treatment with estrogen. Estrogen was active against androgen-stimulated rat tumors which had escaped the effects of castration. Although highly effective, estrogen treatment eventually led to tumor cells which had progressed to an autonomous state that was nonresponsive to other types of hormonal therapy.

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REFERENCES

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