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The biological significance of low testosterone levels and of adrenal androgens in transplantable prostate cancer lines*

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Summary. The transplantable androgen-dependent human prostate tumor models PC-82 and PC-EW were used to study whether low levels of testosterone and androgens of adrenal origin were capable of stimulating the growth of prostatic carcinoma cells in these tumor models. At all circulating plasma testosterone levels applied in this study, much lower levels of dihydrotestosterone were found in PC-EW tumor tissue than in PC-82 tumor tissue. Nevertheless, both prostate tumor models had a similar threshold level of dihydrotestosterone for growth stimulation, i.e. 3–4 pmol/g tissue. This critical androgen level for tumor growth amounted to 2–3 times the tissue level found in castrated animals. At this threshold level approximately 2% of the cells showed proliferative activity, as assessed by bromodeoxyuridine incorporation into DNA. The adrenal androgen dehydroepiandrosterone did not stimulate PC-82 tumor growth, whereas androstenedione did induce a moderate increase in tumor volume. It is concluded that the adrenal androgen androstenedione exerts a stimulating effect on prostatic cancer cells when its conversion results in intra-tissue testosterone and dihydrotestosterone levels exceeding the threshold level for tumor growth.

Key words: Prostate cancer – (Adrenal) androgens – Proliferative activity

The incidence and mortality of prostate cancer in the western world are increasing yearly [1]. Initially, the majority of prostate cancer patients can be treated effectively by endocrine therapy [2, 3]. After variable periods of time, however, all patients relapse due to progression of the tumor from the androgen-responsive stage to an autonomous, uncontrollable state [4]. Standard endocrine therapy, such as surgical or chemical castration, leaves androgens synthesized by the adrenal gland unaffected. It is of clinical importance to know whether adrenal androgens are capable of evoking a proliferative response in prostatic cancer tissue [5].

In this study, the effects of low testosterone (T) levels and of adrenal androgens were studied in the transplantable human tumor models PC-82 and PC-EW. Tumor growth, proliferative activity and intra-tumor androgen levels were studied in hormonally manipulated tumor-bearing mice.

Materials and methods

Tumor model

The PC-82 and PC-EW tumor models are of human origin: the PC-82 tumor is derived from a primary adenocarcinoma of the prostate [6], and the PC-EW tumor originates from a lymph node metastasis of prostatic cancer [7]. Both tumor models can be serially transplanted in athymic nude mice of the Balb/c background. They are dependent on androgens for their growth, as shown by the fact that tumors do not develop in female and castrated male mice [8, 9]. Silastic implants filled with various amounts of different steroids were used for hormonal manipulation of the mice. In order to obtain low circulating levels of T, cholesterol was mixed with various amounts of T [10].

Experimental protocol

The protocol of the experiments is outlined in Fig. 1. Small fragments of tumor were transplanted s.c. in female or castrated male mice supplemented with a T implant, resulting in peripheral T levels of 10 nmol/l, to assure optimal take and tumor growth. Subsequently, mice bearing exponentially growing PC-82 and PC-EW tumors were depleted of androgen for 12 and 5 days, respectively. Finally, mice were resubstituted with various doses of T or with the adrenal androgens, androstenedione and dehydroepiandrosterone (DHEA). Tumor volume was followed weekly by caliper measurements [8]. After 28 days of resubstitution mice were sacrificed and plasma and tumor tissue were sampled.

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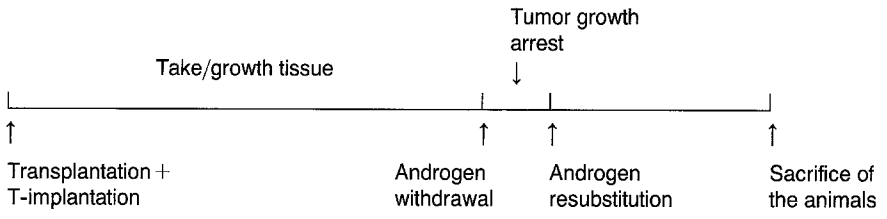

Fig. 1. Protocol of experiments with PC-82 and PC-EW tumor-bearing nude mice substituted with different (doses of) androgens

Table 1. Plasma and tissue levels of testosterone (T) and dihydrotestosterone (DHT) in PC-82 and PC-EW tumor-bearing mice manipulated with various doses of T (Data are expressed as mean ± SEM with number of animals in parentheses)

% T in implant	T plasma (nmol/l)	T tissue (pmol/g)		DHT tissue (pmol/g)	
	PC-82/PC-EW[a]	PC-82	PC-EW	PC-82	PC-EW
0	0.1 ± 0.03 (18)	2.98 ± 0.92 (7)	5.52 ± 6.54 (6)	1.28 ± 0.77 (7)	1.26 ± 1.10 (6)
5	0.6 ± 0.2 (12)	11.61 ± 5.08 (17)	8.21 ± 7.47 (6)	6.12 ± 3.55 (17)	2.18 ± 2.07 (6)**
10	1.3 ± 0.3 (17)	19.07 ± 8.70 (10)	7.95 ± 6.17 (6)*	9.24 ± 4.86 (10)	3.65 ± 2.68 (6)**
25	4.5 ± 1.0 (4)	–	20.62 ± 3.03 (4)	–	5.17 ± 0.97 (4)
100	15.6 ± 1.3 (8)	33.61 ± 4.05 (8)	25.45 ± 2.25 (3)*	20.22 ± 4.89 (8)	8.21 ± 1.72 (3)**

[a] Plasma T levels were not significantly different in the two tumor groups
* Significantly different from PC-82 data (Students t-test, $P < 0.05$)
** Significantly different from PC-82 data (Students t-test, $P < 0.001$)

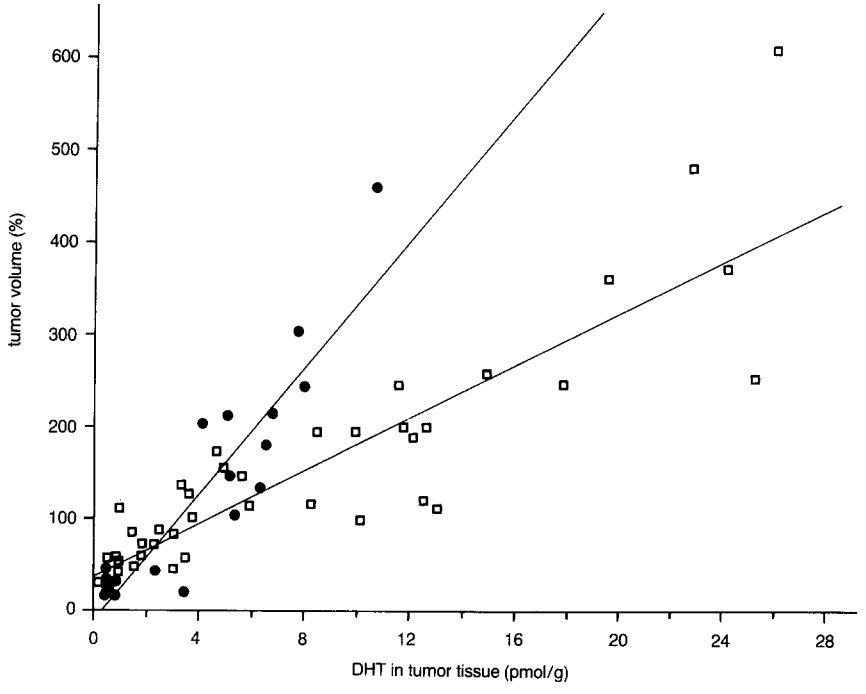

Fig. 2. Correlations between intratissue DHT content and growth of the PC-82 (□) and PC-EW (●) tumors in female nude mice substituted for 28 days with various doses of T

Hormone estimations

Endogenous T and dihydrotestosterone (DHT) were determined in whole-tissue homogenates after separation of the androgens on silica columns as described by Hämäläinen et al. [11]. Tissue T and DHT and plasma T concentrations were estimated by radioimmunoassay [12].

Assessment of proliferative activity

One hour prior to sacrifice mice received the thymidine analogue bromodeoxyuridine (BrdU) 10 mg/kg i.p. BrdU incorporation into DNA was visualized by an anti-BrdU monoclonal antibody using an indirect peroxidase staining procedure [13]. BrdU-positive cells were counted and expressed as percentage of the total number of cells (1,000) counted per sample.

Results

Androgen levels and tumor growth

Substitution of tumor-bearing mice with different doses of T resulted in mean plasma T levels ranging from 0.1 to 15.6 nmol/l (Table 1). The corresponding intra-tissue T

and DHT concentrations of PC-82 and PC-EW tumors are also summarized in Table 1. Within all substitution groups T levels in PC-EW tumor tissue were slightly lower than those found in PC-82 tumors, whereas the levels of DHT in PC-EW tumors were between one-third and half the levels found in PC-82 tumors. In animals receiving cholesterol only (0%T) castrate androgen levels in both types of tumors were similar. Correlation of intra-tissue DHT levels with tumor growth after 28 days of substitution ($r=0.88$, $n=39$, $P<0.001$, and $r=0.91$, $n=18$, $P<0.001$ for PC-82 and PC-EW, respectively; Fig. 2) clearly showed that the slope of the regression line for the PC-EW tumor was significantly steeper than that for the PC-82 tumor (slope: 36.2 ± 2.7 and 14.5 ± 1.3, respectively). In the PC-EW tumor maximum concentrations of DHT were 10 pmol/g, whereas in the PC-82 tumor DHT levels could exceed 20 pmol/g tissue. In spite of these differences, in neither tumor model did any growth at all occur at DHT concentrations lower than approximately 3 pmol/g tissue, indicating that both tumors need the same intratissue concentration of androgen for their growth.

Proliferative activity

The proliferation index in PC-82 tumor tissue, assessed by BrdU incorporation into DNA of the cells, significantly correlated with intratissue DHT content ($r=0.80$, $n=23$, $P<0.001$) (Fig. 3). In the PC-EW tumor these parameters were not significantly correlated ($r=0.40$, $n=15$). Below the threshold level of 3 pmol DHT/g tissue about 1–2% of cells still incorporated BrdU into DNA.

Adrenal androgen substitution

Substitution of mice with androstenedione and DHEA resulted in tumor growth of the PC-82 tumor only in those mice substituted with androstenedione (Fig. 4). In DHEA-treated animals (plasma level: 9.2 ± 3.9 nmol/l), PC-82 tumor growth, intratissue T and DHT levels were found to be similar to those observed in castrated animals. In contrast, substitution of androstenedione (plasma level: 10.9 ± 2.9 nmol/l), resulting in a submaximal tumor growth (217% vs 362% in T-substituted mice), caused T and DHT tissue levels between those found in 10% and 100% T-substituted mice (results now shown).

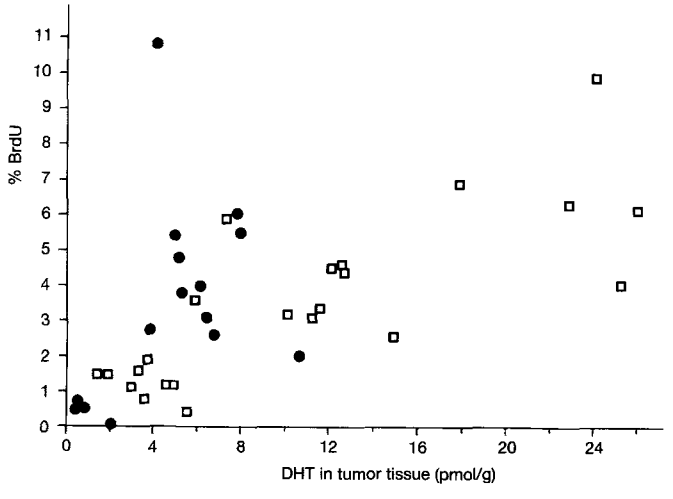

Fig. 3. Proliferative activity of PC-82 (□) and PC-EW (●) tumor tissue at different intratumor concentrations of DHT as assessed with the BrdU labelling technique

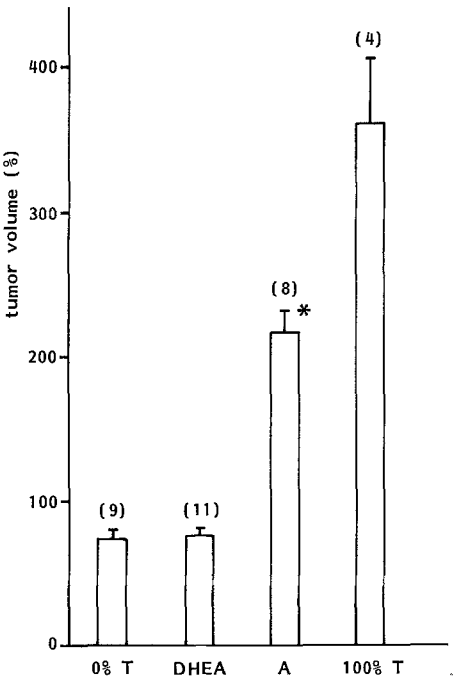

Fig. 4. The effect of the adrenal androgens androstenedione and DHEA on growth of the PC-82 tumor after 28 days of substitution. Results are expressed as percentages of tumor volume at the start of treatment. * Significantly different from all substitution groups (Student's *t*-test, $P<0.05$)

Discussion

The process of progression of prostatic carcinoma to a hormone-refractory autonomous state is still poorly understood and the main difficulty in the treatment of prostate cancer patients remains the relapse of cancer growth after anti-hormonal therapy. It has been shown by many investigators that castration causes only a partial depletion of tissue androgens [14, 16]. Whether remaining low levels of androgens (possibly of adrenal origin) could exert the proliferative stimulus inducing the growth relapse of the regressed prostatic carcinoma is still a matter of debate [17]. In this study, the PC-82 and PC-EW prostate tumor models of human origin have been used to investigate the relationship between low levels of intratissue androgens and tumor growth.

Substitution of tumor-bearing mice with various doses of T resulted in intratissue concentrations of DHT ranging from castrate levels (1 pmol/g tissue) up to maximal levels of DHT in prostate tumor tissue of 30 and 10 pmol/g tissue for the PC-82 and PC-EW tumor,

respectively. By correlating intratumor DHT content with tumor growth it was demonstrated that a critical level of DHT of approximately 3 pmol/g tissue is needed in both tumor models to stimulate tumor growth. The fact that this threshold of DHT was found to amount to 2–3 times the level found in castrated animals (1 pmol DHT/g tissue) indicates that additional withdrawal of androgens seems not necessary to stop growth of these tumor models, although only castration (0% T) resulted in a maximal decline in tumor volume [18, 19]. These data confirm those obtained from studies with the Dunning rat prostate tumor model system: hormonal titration of plasma levels of T showed that a threshold level existed below which the Dunning R3327-H tumor is inhibited from growing [20]. Ellis and Isaacs [21] observed similar results using both the G and H subline of the Dunning R3327 system. These authors concluded that serum T levels should be maintained below 1.7 nmol/l but did not have to be completely eliminated to produce the maximum therapeutic response, i.e., reduction of tumor volume similar to that seen in castrates. In addition, using the rat ventral prostate (RVP), an increase in prostatic cell number was detected only when the concentration of prostatic DHT exceeded a critical threshold value (0.4 ng/10^8 cell RVP). Stimulation of growth could, thus, be eliminated just by lowering the prostate DHT content below this critical threshold [22]. Moreover, in the Dunning H and G sublines complete androgen withdrawal consisting of surgical castration in combination with daily treatment with CPA was no more effective in terms of tumor growth retardation than was partial androgen withdrawal induced by castration alone [20]. This observation probably also relates to the existence of an androgen threshold for tumor growth in this model system.

It is tempting to speculate about the significance of the remaining part of cells still capable to incorporate BrdU (1–2%) in regressing tumors. This finding suggests that a small fraction of cells proliferates at androgen levels below the critical level needed for growth. Alternatively, it may also represent a background staining due to DNA repair rather than DNA replication [23]. As the PC-82 and PC-EW tumor models have never shown a spontaneous relapse of tumor growth in the absence of androgens [8] such as seen in the Dunning system [24], the remaining proliferation cannot simply be explained by the presence of androgen-independent tumor cells.

If a threshold level for tumor growth exists, what then is the relevance of androgens secreted from the adrenal glands? It is known that the human adrenal glands secrete considerable amounts of the androgens androstenedione and DHEA [25]. These androgens can be converted in the peripheral tissues as well as in the prostate into the active androgens T and DHT [26]. A compensatory increase in adrenal androgen output following orchidectomy could not be found in studies on serum levels of androstenedione and DHEA in patients with prostate cancer [27, 28]. While adrenal androgens are not capable of supporting development and growth of the normal prostate in men [29], other investigators found stimulation of growth by both androstenedione and DHEA of the normal rat ventral prostate [30–32]. These investigators showed an increase in weight of the ventral prostate, of prostatic DHT content and of mRNA levels encoding specific androgen-regulated proteins in androstenedione- and DHEA-substituted castrated male rats. The stimulatory effect of androstenedione was more pronounced than that of DHEA. In contrast to these data, no effect of DHEA on PC-82 tumor growth or on DHT levels in the tumor tissue could be detected. Whether this discrepancy is due to the differences between normal and tumor tissue or to species (i.e., rat vs man) differences in metabolic capacity remains to be investigated. The former possibility is supported by the observation that lower activities of hormone-dependent enzymes were observed in untreated cancers of the prostate than in the normal prostate [33], suggesting a less efficient utilization of steroid hormones. Androstenedione resulted in suboptimal growth of the PC-82 tumor. The plasma levels of androstenedione in the mice (approximately 11 nmol/l) were about twice the levels of 4–5 nmol/l normally found in hormonally treated prostate cancer patients (F. H. de Jong, unpublished results). It remains to be seen whether such levels of androstenedione are stimulatory for the PC-82 tumor as well. A detailed description and analysis of the effect of adrenal androgens will be the subject of a separate publication.

It has been shown very recently [34] that 31 of a group of 38 castrated prostate cancer patients had residual DHT levels below 4 pmol/g tissue. If these data are compared with the observations made with the PC-82 and PC-EW tumor models, then one might conclude that total androgen blockade does not add to tumor growth inhibition in most of the patients. Interestingly, however, in 7 of the 38 patients DHT levels up to 10 pmol/g tissue could still be detected. In 1 patient even the relatively high level of 16.5 pmol DHT/g tissue was found (J. Geller, personal communication). These levels are in excess of the critical level for growth described above, and tumor growth may thus be expected to be stimulated. According to the data obtained with the PC-82 tumor after adrenal androgen substitution, high levels of prostatic DHT originate mainly from conversion of androstenedione rather than from DHEA. It remains unknown, however, whether the high levels of prostatic DHT found in some prostate cancer patients are due to elevated levels of androstenedione.

The present results obtained with the PC-82 and PC-EW tumor model systems yield information on the effect of androgens on human prostate tumor growth. In conclusion, the data presented in this paper indicate that submaximal suppression of androgens can stop tumor growth. Maximal tumor regression is seen only with extremely low androgen levels. When relatively high levels of androstenedione remain in circulation tissue DHT levels may rise and lead to stimulation of tumor growth. Amounts of androstenedione used in these experiments have not been found in patients receiving standard endocrine treatment. The high DHT tumor tissue levels described after castration in some patients can, however, be explained by adrenal androgen production.

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