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Development of Androgen-independent Carcinomas from Androgen-dependent Preneoplastic Lesions in the Male Accessory Sex Organs of Rats Treated with 3,2′-Dimethyl-4-aminobiphenyl and Testosterone Propionate

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Two kinds of cancer can be induced in rat male accessory sex organs, one a non-invasive carcinoma arising in the ventral lobe and the other an invasive lesion which develops in the dorsolateral and anterior lobe as well as the seminal vesicles. In the present study, one group of male rats were given biweekly s.c. injections of 3,2′-dimethyl-4-aminobiphenyl (DMAB) for 20 weeks for induction of non-invasive carcinomas and the other group received DMAB with 40-week testosterone propionate for induction of invasive carcinomas. Half of the animals in each group were then subjected to bilateral orchiectomy at week 41 to remove testicular androgen, in order to examine the androgen dependence of both types of carcinomas as well as precancerous lesions. Animals were killed at weeks 41, 46 and 60. All parts of the prostate complex showed involution and significant weight reduction after castration, with a complete disappearance of atypical hyperplasias and carcinomas of the ventral prostate. However, in spite of suppression of development of atypical hyperplasias in the anterior prostate and seminal vesicles, the incidence of invasive carcinomas was not changed. Normal epithelial cells and atypical hyperplasias of all parts of the prostate and seminal vesicles and carcinomas of the ventral prostate were immunohistochemically positive for nuclear androgen receptor, while invasive carcinomas that developed in either castrated or non-castrated animals were negative. These findings suggest that in the ventral prostate, both precancerous and cancerous lesions are androgen-dependent, but in the anterior and seminal vesicles, cancerous lesions (invasive carcinomas) are androgen-independent while precancerous lesions are hormone-dependent.

Key words: Androgen-independent carcinoma — Rat — Prostate

The prostate glands require androgens for their growth, maintenance and function. Furthermore, development of tumors of the prostate is strongly related to the sex hormone status. For example, men castrated when young are reported not to develop prostate cancer.[1)] Deficiency of 5α-reductase, the enzyme which converts testosterone into the dihydrotestosterone active form in target organs is also linked to low susceptibility.[2, 3)] It is well known that prostate cancers are controllable by androgen deprivation therapy in early stages, but that androgen dependence is later lost, so lesions become incurable with hormone therapy.

In experimental models, pharmacological doses of testosterone have been shown to increase development of naturally occurring or chemically-induced prostate carcinomas in rats.[4–6)] We have reported that chronic administration of testosterone propionate (TP) at a pharmacological dose after 3,2′-dimethyl-4-aminobiphenyl (DMAB) induces invasive and partly metastatic adenocarcinomas, arising from the dorsolateral and anterior lobes, as well as the seminal vesicles.[7)] Our previous work with immunohistochemical analysis of androgen receptors showed most ventral prostate carcinomas to be positive while more than 80% of invasive carcinomas arising from the dorsolateral prostate and seminal vesicle proved negative.[8)] Since normal glandular epithelial cells and atypical hyperplasias, regardless of the location in the accessory sex organs, are positive for androgen receptor (AR) immunohistochemistry, alteration in AR signalling might be a critical step for progression from atypical hyperplasias to invasive carcinomas. Orchiectomy after 20-week administration of testosterone to DMAB-treated animals resulted in development of a few cases of invasive carcinomas by the end of the experiment, suggesting that androgen-independent lesions can indeed be induced.[9)]

In the present study, the appearance of typical hyperplasias and carcinomas of the prostate and seminal vesicles after castration of rats was analyzed in order to clarify their androgen dependence.

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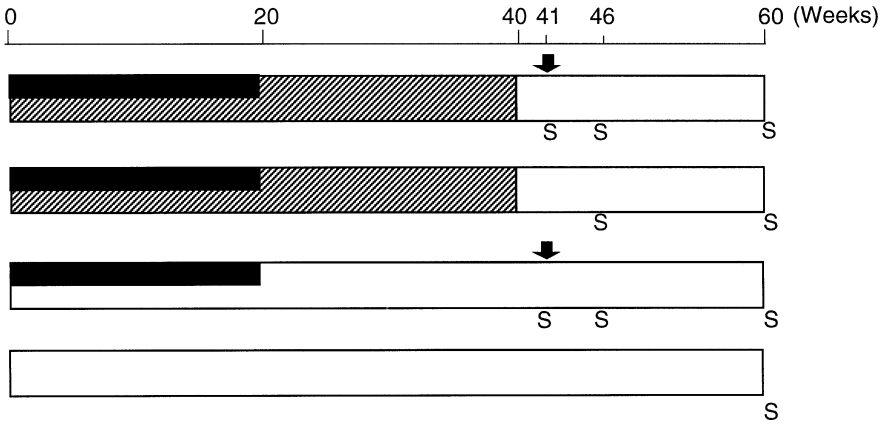

Fig. 1. Experimental design. Animals used were male F344 rats 6 weeks old at the commencement. DMAB, 3,2′-dimethyl-4-aminobiphenyl; b.w., body weight; s.c., subcutaneous. Animals: F344 male rats, 6 weeks old at the commencement. ■, DMAB 50 mg/kg b.w. s.c. biweekly; ▨, testosterone propionate, Silastic tube (40 mg); ⬇, castration; S, animal killed.

MATERIALS AND METHODS

Chemicals DMAB was obtained from NARD Institute (Amagasaki) and its purity was above 98%. TP was purchased from Tokyo Kasei (Tokyo).

Animals A total of 120 male F344 rats (5 or 6 weeks old) were purchased from Charles River Japan (Atsugi) and housed three to a plastic cage, on hard-wood chips in an air-conditioned room at 22±2°C and 50% humidity with a 12 h-12 h light-dark cycle. They were given commercial pellets (Oriental MF; Oriental Yeast Co., Ltd., Tokyo) and tap water *ad libitum*.

Experimentation The animals were divided into 4 groups as shown in Fig. 1 (45, 35, 35 and 10 rats for groups 1 to 4, respectively). The animals in groups 1 to 3 were given DMAB s.c. at a dose of 50 mg/kg body weight 10 times at 2-week intervals. Those in groups 1 and 2 also received a pharmacological dose of TP from the beginning of the experiment for 40 weeks (until week 40), applied in Silastic tubes (2-cm long with 3 mm outer diameter and 2 mm inner diameter containing approximately 40 mg of TP), implanted into the subcutis every 6 weeks. Methods for administration of DMAB and TP were the same as reported previously.[7, 10, 11] Ten animals of group 1 and 8 of group 3 were killed at week 41 (one week after the cessation of treatment with TP), and the remaining rats were subjected to bilateral orchiectomy under ether anesthesia. Subgroups of 5 to 10 rats in groups 1 to 3 were killed at week 46. The experiment was terminated at experimental week 60 when all surviving animals were killed.

All rats underwent complete autopsy. All accessory sex organs were examined for gross abnormalities and fixed in 10% buffered formalin. For tissue preparation, 2 sagittal slices of the ventral prostate, 3 sagittal samples of the dorsolateral prostate, including the urethra, and 4 transverse samples from each seminal vesicle, including the anterior prostate (coagulating glands), were embedded in paraffin. Sections were cut and stained with hematoxylin and eosin for histopathological examination.

AR immunohistochemistry was carried out by the methods described previously.[8] After deparaffinization, rehydration and heating in a microwave oven following washing with phosphate-buffered saline, the sections were treated with 2% goat serum at 4°C to block nonspecific binding, and then incubated overnight at 4°C with the polyclonal rabbit antibody PG21[12] at a concentration of 2.0 μg IgG/ml. Binding was visualized by the avidin-biotin-peroxidase complex method with 0.05% 3,3′-diaminobenzidine. The sections were then counterstained weakly with hematoxylin. Negative control sections were processed in an identical manner with substitution of normal rabbit IgG for the primary antibody.

Differences in body and organ weights were analyzed by means of Student's *t* test. Incidences of tumors and other histopathological lesions were analyzed by using Fisher's exact probability test (two tailed).

RESULTS

Administration of DMAB did not markedly alter the growth rate of rats but treatment with TP retarded their growth (about 20% suppression of body weight increase). After cessation of TP treatment, body weight gain was increased, though it was slightly suppressed by castration (group 1). Castration of DMAB alone-treated rats decreased their body weights by about 25 g (to 94% of the body weight expected at week 42) (Fig. 2).

Table I summarizes data for prostate and seminal vesicle weights. One week after the cessation of TP treatment, weights returned to the control values (group 1 vs. group 3), while castration induced marked reduction in the weights in both groups 1 and 3 (lesser degree in group 1, statistically significant at weeks 46 and 60 at $P<0.01$ and 0.001). Castration induced a gradual decrease in prostate and seminal vesicle weights over 20 weeks and at week 60, those for ventral and dorsolateral lobes were reduced to about 1/3 to 1/4 and that of the seminal vesicles to about 1/10 of the respective control values.

The preneoplastic and neoplastic lesions of the accessory sex organs were histologically classified into atypical hyperplasias and adenocarcinomas as in previous studies[7, 13] (Figs. 3–5). The incidences are summarized in Table II.

In the animals given DMAB and TP, atypical hyperplasias of the ventral, dorsolateral and anterior prostate and seminal vesicles (Figs. 3–5) at week 41 were observed at incidences of 13%, 38%, 75% and 75%, respectively (Table II). That for the ventral prostate was marginally but significantly lower than for rats given DMAB alone ($P<0.07$). After cessation of TP treatment, there was a tendency for the atypical hyperplasias of the ventral prostate and seminal vesicles to increase. Two cases of invasive carcinomas were observed at week 60 (one each of anterior prostate and seminal vesicles).

Castration, regardless of pretreatment with carcinogen and TP, markedly suppressed atypical hyperplasias in all regions (Table II). Although administration of DMAB alone was associated with high incidence of atypical hyperplasias in the ventral prostate and seminal vesicles (week 41), castration completely blocked development of such lesions ($P<0.05$ and 0.001, respectively). In rats given DMAB and TP, atypical hyperplasias of the anterior prostate and seminal vesicles were also significantly suppressed by castration ($P<0.05$). Interestingly, one of the castrated rats given DMAB plus TP developed a large tumor mass involving the anterior prostate and seminal

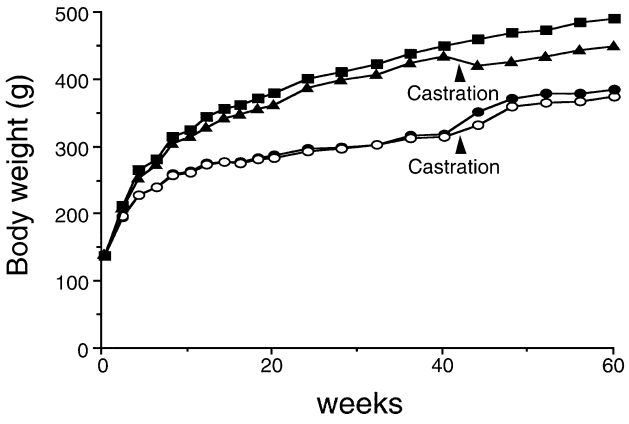

Fig. 2. Growth curves of rats given DMAB with/without TP and castration. ○, DMAB+TP→castration; ●, DMAB+TP; ▲, DMAB→castration; ■, no treatment.

Table I. Changes in Prostate and Seminal Vesicle Weights

Group	Treatment	Experimental time points		
		41 wks (Before CX)	46 wks	60 wks
Ventral prostate				
1	DMAB+TP →CX	0.51±0.12 (8)	0.23±0.05 (10) [a,c]	0.13±0.03 (13) [a,d]
2	DMAB+TP	0.51±0.12 (8)	0.56±0.09 (8)	0.58±0.15 (13)
3	DMAB →CX	0.55±0.10 (10)	0.14±0.04 (5)	0.06±0.01 (13)
4	No treatment	NE	NE	0.50±0.06 (10)
Dorsolateral prostate				
1	DMAB+TP →CX	1.05±0.10 (8) [d]	0.40±0.05 (10) [a,d]	0.25±0.04 (13) [a,d]
2	DMAB+TP	1.05±0.10 (8) [d]	0.87±0.06 (8)	0.91±0.15 (13)
3	DMAB →CX	0.70±0.05 (10)	0.26±0.03 (5)	0.18±0.02 (13)
4	No treatment	NE	NE	0.67±0.10 (10)
Seminal vesicles with anterior prostate				
1	DMAB+TP →CX	0.93±0.42 (8)	0.24±0.04 (10) [a,b]	0.15±0.02 (13) [a,d]
2	DMAB+TP	0.93±0.42 (8)	1.24±0.27 (8)	1.40±0.45 (13)
3	DMAB →CX	1.02±0.18 (10)	0.18±0.03 (5)	0.11±0.01 (13)
4	No treatment	NE	NE	1.11±0.10 (10)

( ) : number of rats. CX: castration. NE: not examined.
a) Significantly different from group 2 at $P<0.001$.
b, *c*, *d*) Significantly different from group 3 at $P<0.05$, $P<0.01$, $P<0.001$, respectively.

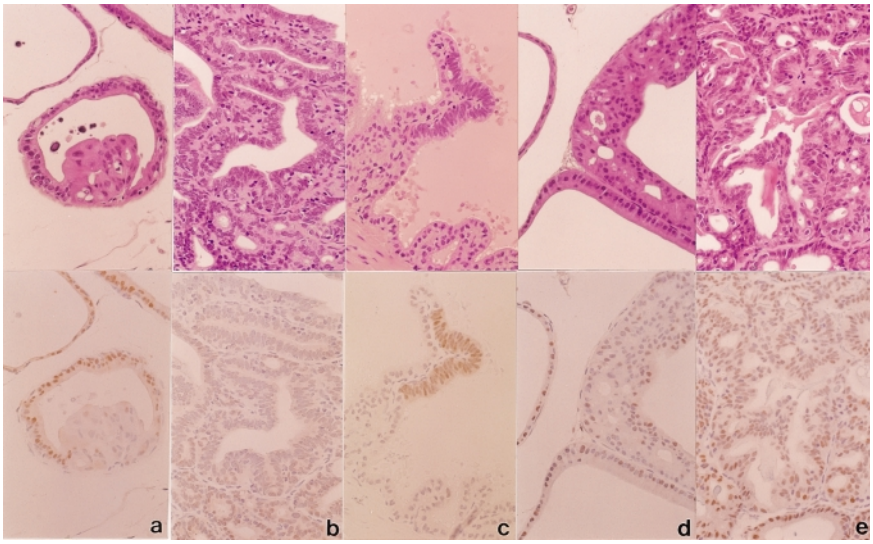

Fig. 3. Histopathological appearance and AR immunostaining of atypical hyperplasias of the prostate and seminal vesicles of rats at week 41 just before castration. Fig. 3a–c are for DMAB and TP treatment (group 1) and Fig. 3d and e for DMAB alone (group 3). The upper sections are H.E.-stained (×200) and the lower ones illustrate corresponding AR immunohistochemistry (×200). a and d, ventral prostate; b and e, seminal vesicle; c, anterior prostate.

vesicles. It was diagnosed as a seminal vesicle carcinoma on histopathological analysis. No metastasis was present.

AR immunohistochemistry Table III summarizes the results for immunohistochemical staining intensity in the nuclei of the epithelial components of non-tumor and tumorous regions of the prostate and seminal vesicles. The data for alteration in AR staining with age and castration are included.

No clear changes in nuclear staining intensity of either normal epithelium or atypical hyperplasias were observed between TP-treated and TP-untreated groups before orchiectomy or between weeks 41 and 60 in group 2 (Figs. 3 and 5). All atypical hyperplasias of the ventral and dorsolateral prostate and seminal vesicles were positive for AR immunostaining but less intensely stained than the normal epithelium. Immunostaining of those of the anterior prostate however, was increased as compared to that of the normal epithelium (Figs. 3c and 5d). Castration at week 41 reduced the volume of epithelial structures (as a result of involution of glands) and the AR immunostaining intensities in all parts examined at weeks 46 and 60 (Fig. 4). As demonstrated in Fig. 4, positive signals were still seen in the cytoplasm as well as nuclei of the epithelial cells after castration. Ventral prostate carcinomas at week 60 all demonstrated strongly positive nuclei.[8] Evaluation of AR staining in atypical hyperplasias at weeks 46 or 60 in castrated groups was available only for the anterior prostate and seminal vesicles, castration not causing any change in staining intensity. While atypical hyperplasias of the anterior prostate and seminal vesicles were positive for AR immunostaining, invasive carcinomas of the anterior prostate or seminal vesicles developing in either castrated or non-castrated animals which had been treated with DMAB and TP were completely negative (Fig. 5).

DISCUSSION

The ventral lobe of the rat prostate appears to be biologically different from the other lobes and the seminal vesicles, because ventral glands show marked cystic and atrophic changes with age, whereas the others do not, even though all parts of the male accessory sex organs are dependent on androgen for their growth and function.[14] A clear decline of serum testosterone levels is not observed before 70 weeks of age in the rat.[6, 15] The lobe-specific change in rats may mimic the situation in the prostate of aged men, where glands of the peripheral zone become atrophic while those of the transition zone rather exhibit hyperplasia. However, unlike the human case, in the rat prostate, non-invasive and non-metastasizing prostate carcinomas preferentially develop in the ventral lobe, while invasive and metastatic lesions appear in the dorsolateral and anterior lobes and seminal vesicles.[7, 11, 13, 15] The former are produced by administration of DMAB alone while the latter arise after combined administration of DMAB and a pharmacological dose of TP.

The present experiment was carried out to examine the androgen-dependence of preneoplastic and neoplastic lesions of rat prostate and seminal vesicles of rats given

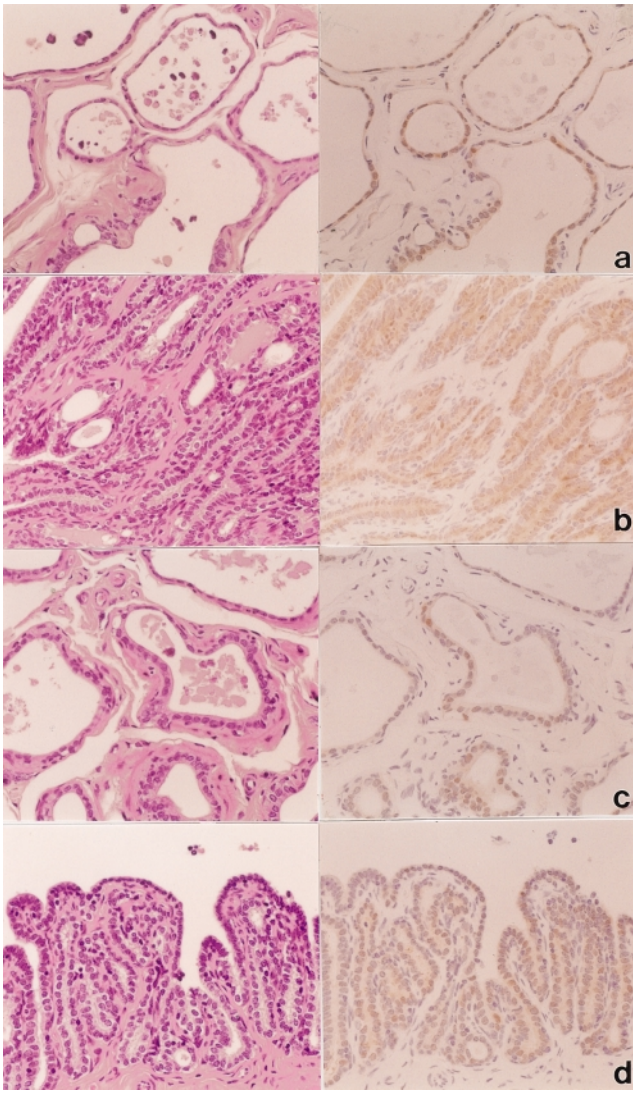

Fig. 4. Histopathological appearance and AR immunostaining of atypical hyperplasias of the prostate and seminal vesicles of rats given DMAB plus TP (Fig. 4, a and b) or DMAB (Fig. 4, c and d), 5 weeks after castration. Fig. 4, a and c, ventral prostate; Fig. 4, b and d, seminal vesicles. Sections on the left are H.E.-stained (×200) and those on the right illustrate corresponding AR immunohistochemistry (×200).

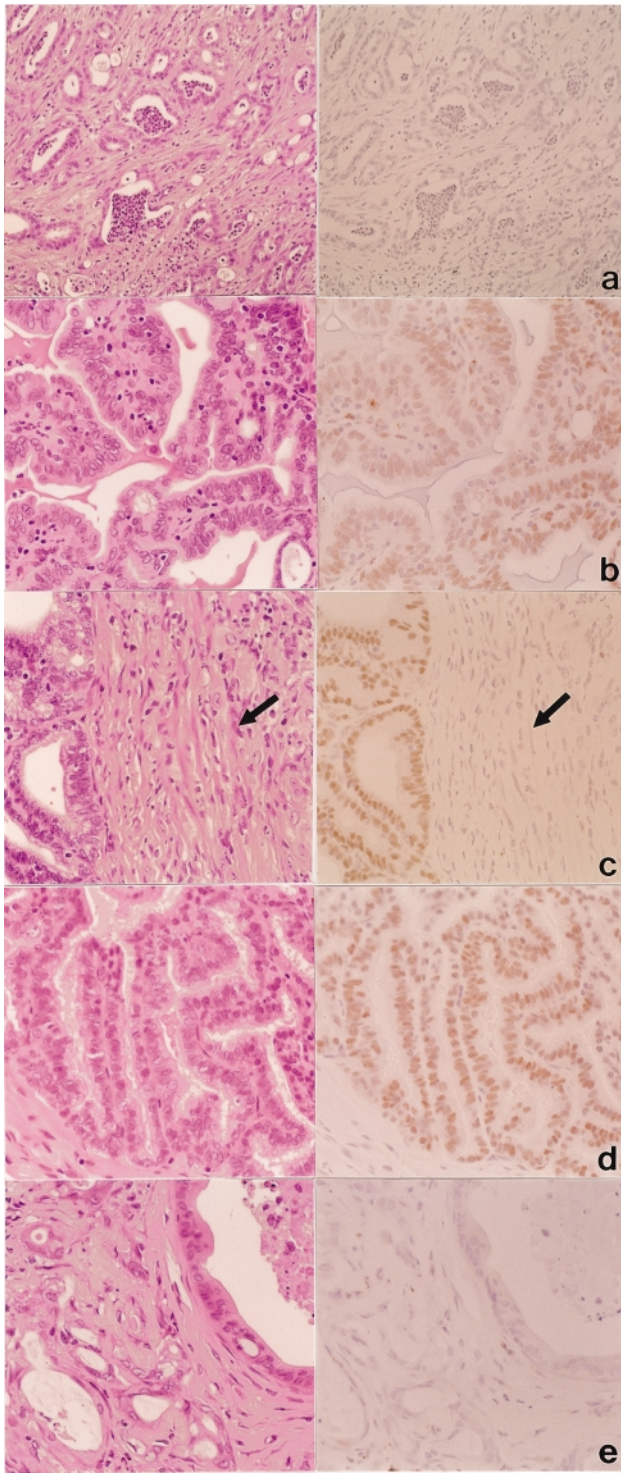

Fig. 5. Histopathological appearance and AR immunostaining of atypical hyperplasias and carcinomas of the prostate and seminal vesicles of rats given DMAB plus TP with (Fig. 5a) or without (Fig. 5, b–e) castration. Sections on the left are H.E.-stained (×400) and those on the right illustrate corresponding AR immunohistochemistry (×400). a, advanced seminal vesicle invasive carcinoma ; b, seminal vesicle atypical hyperplasia; c, early invasive carcinoma of the seminal vesicle (arrows); d, anterior prostate atypical hyperplasia and e, invasive carcinoma of the anterior prostate.

Table II. Incidence of Carcinomas (Ca) and Atypical Hyperplasia (AH) in the Prostate and Seminal Vesicles

Treatment	Experimental time points	No. of rats	Ventral		Dorsolateral		Anterior		Seminal vesicle	
			AH (%)	Ca (%)	AH (%)	Ca (%)	AH (%)	Ca (%)	AH (%)	Ca (%)
DMAB+TP	41 (wks)[a]	8	1 (13)	0	3 (38)[e]	0	6 (75)[f, g]	0	6 (75)[h, i]	0
→Cx	46	10	0	0	0	0	1 (10)[f, g]	0	1 (10)[h]	0
	60	13	0[d]	0	0[e]	0	0[g]	0	0[i]	1 (8)
DMAB+TP	41[a]	8	1 (13)	0	3 (38)[j]	0	6 (75)	0	6 (75)	0
	46	8	1 (13)	0	0	0	5 (63)	0	8 (100)	0
	60	13	8 (62)[d]	0	0[j]	0	7 (54)	1 (8)	13 (100)	1 (8)
DMAB→Cx	41[b]	10	6 (60)[k, l]	0	0	0	1 (10)	0	10 (100)[m, n]	0
	46	5	0[k]	0	0	0	0	0	0[m]	0
	60	13	0[l]	0[o]	0	0	0	0	0[n]	0
DMAB	41[b]	10	6 (60)[p]	0	0	0	1 (10)	0	10 (100)	0
	60[c]	59	50 (85)	22 (37)[o]	1 (2)	0	1 (2)	0	55 (93)	0
No treatment	60	10	0	0	1 (10)	0	0	0	0	0

TP : testosterone propionate. Cx : castration
a, *b*) The same data as before orchiectomy.
c) Data for DMAB 60 wks are historical results (from refs. 10, 16 and 17).
Statistically significant differences exist between values with the same superscript: $P<0.05$ (*e*, *j*, *k*, *l*); $P<0.02$ (*f*, *h*); $P<0.01$ (*d*, *o*); $P<0.001$ (*g*, *i*, *m*, *n*).

Table III. Findings for Androgen Receptor Immunohistochemistry

Treatment	41 wks									60 wks									
	V		DL		A		SV			V		DL		A			SV		
	N	AH	N	AH	N	AH	N	AH		N	AH	N	AH	N	AH	CA	N	AH	CA
DMAB + TP	++	±	++	+	±	++	++	±	Castration →	±	NA	±	NA	±	NA	NA	+	NA	–
									Non-castration →	++	+	++	NA	±	++	–	++	±	–
DMAB	++	±	++	NA	±	++	++	+	Castration →	±	NA	±	NA	±	NA	NA	±	NA	NA

Staining intensity: –, negative; ±, weakly positive; +, positive; ++, strongly positive.
V, ventral prostate; DL, dorsolateral prostate; A, anterior prostate; SV, seminal vesicles; N, normal; AH, atypical hyperplasia; CA, carcinoma; NA, not available.

DMAB with/without TP. For comparison, historical data on the development of prostate carcinomas and atypical hyperplasias of the seminal vesicles in rats given DMAB alone at a dose of 50 mg/kg 10 times were taken from three published experiments[10, 16, 17] (Table II). Historically, DMAB alone induced 85%, 37% and 93% incidences of atypical hyperplasias and carcinomas of the ventral prostate and atypical hyperplasias of the seminal vesicles, respectively. Castration of animals after the carcinogen treatment with/without TP completely suppressed the appearance of atypical hyperplasias in these sites without affecting the incidences of invasive carcinomas in the anterior prostate and seminal vesicles. The present data confirmed our previous finding that a few invasive carcinomas develop in the involuted accessory sex organs of rats treated with DMAB and TP and subsequently orchiectomized.[9]

In accordance with the androgen-dependent growth and function of all parts of the accessory sex organs, nontumorous epithelial cells were immunohistochemically found to be strongly positive for AR, as observed in a previous study.[8] It is important to note that all atypical hyperplasias examined were more or less positive. Our findings on the incidences of the lesions and AR immunostaining indicate that atypical hyperplasias and carcinomas of the ventral prostate and atypical hyperplasias, but

not invasive carcinomas of the anterior prostate and seminal vesicle, are androgen-dependent.

The number of lesions was unfortunately very limited in groups undergoing orchiectomy, so that an in-depth analysis of AR status at weeks 41 and 60 was not possible. It is noteworthy, however, that AR expression in invasive carcinomas in both castrated and non-castrated groups was negative. Since no carcinomas were present at the time of castration (week 41), it was not possible to determine changes in AR status of carcinomas after withdrawal of TP or due to castration.

AR abnormalities have been implicated in conversion from androgen-dependent to -independent, hormone-refractory cancers in man. Mutations of the *AR* gene have been detected in only rather small proportions of recurrent prostate cancers examined.[18–22] There are several lines of evidence showing that *AR* gene amplification can contribute to hormone-independent growth of advanced prostate cancers in man.[23–25] Recently we found that regulatory regions of the *AR* gene of three transplantable prostate cancer cell lines established from prostate invasive carcinomas induced by DMAB and testosterone[26] were hypermethylated (unpublished data). Thus, hypermethylation of the *AR* gene is one possible mechanism of acquisition of androgen-independent growth of DMAB- and TP-induced carcinomas. It is of great interest that induction of invasive carcinomas in the accessory male sex organs of rats requires long-term administration of high doses of testosterone, but that most of the carcinomas lose their AR and show androgen-independent growth. We speculate that pharmacological testosterone stimulation disturbs AR or signalling status and that this facilitates acquisition of invasiveness.

In conclusion, carcinomas of the ventral prostate are androgen-dependent but some of those induced by DMAB with a high dose of testosterone become androgen-independent. The present model provides a good experimental tool for investigation of the mechanisms underlying relationships between androgen function and testosterone treatment as well as their significance for the development of different types of cancer. Further large-scale experiments are now required to demonstrate whether or not acquisition of invasive growth by carcinomas requires loss of androgen dependence in experimental prostate carcinogenesis.

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