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Animal Models for the Study of Prostate Carcinogenesis

Maarten C. Bosland, D.V.S., Ph.D.

Institute of Environmental Medicine, New York University Medical Center, Tuxedo, New York 10987

Abstract Human prostate carcinogenesis has been viewed as a multi-step process involving progression from low histologic grade, small, latent carcinoma to large, higher grade, metastasizing carcinoma. However, recent data suggest that a variety of pathogenetic pathways may exist. The precise etiology and pathogenesis of human prostate cancer remain largely undefined. It is difficult to investigate stages in the development of human prostate cancer, but some animal models provide opportunities in this regard. Short-term treatment of rats with chemical carcinogens produces a low incidence (5-15%) of prostate cancer, provided that prostatic cell proliferation is enhanced during carcinogen exposure. Chronic treatment with testosterone also produces a low prostate carcinoma incidence. A high carcinoma incidence can only be produced by chronic treatment with testosterone following administration of carcinogens such as *N*-methyl-*N*-nitrosourea (MNU) and 3,2'-dimethyl-4-aminobiphenyl (DMAB). Testosterone markedly enhances prostate carcinogenesis even at doses that do not measurably increase circulating testosterone. Thus, testosterone is a strong tumor promoter for the rat prostate. All such MNU- or DMAB-initiated and/or testosterone-promoted tumors are adenocarcinomas; most originate from the dorsolateral and anterior, but not ventral, prostate lobes. These tumors share a number of important characteristics with human prostate cancer. A high frequency (70%) of activation of the K-*ras* gene by a G^{35} to A mutation occurs in these carcinomas. Another high incidence prostate carcinogenesis model, representing a different pathogenetic pathway, involves chronic administration of estradiol-17β to rats in combination with low-dose testosterone. The resulting carcinomas are low-grade and originate exclusively from periurethral ducts of the dorsolateral and anterior prostate. While it is unknown whether testosterone is a tumor promoter in this system, preliminary studies indicate the formation of a DNA adduct in the target tissue, which suggests that estradiol-17β acts as a tumor initiating agent in this system. The high incidence models mentioned earlier are adequate for the study of chemoprevention of prostatic carcinogenesis. Analysis of shifts in the relative incidence of metastasizing carcinoma, grossly apparent but not-metastasizing carcinoma, microscopic-size carcinoma, and carcinoma *in situ* or atypical hyperplasia may allow study of the modifying effects of potential chemopreventive agents on tumor progression in these animal models of prostatic carcinogenesis.

Key words: chemoprevention, DMAB, estradiol, MNU, prostate, rat, testosterone

Cancer of the prostate is the most frequently diagnosed cancer and the second most frequent cause of death due to cancer in males in the USA and many west European countries [1,2]. Notwithstanding the clinical importance of prostate cancer, its etiology is poorly understood [2]. Environmental factors are probably critical determinants of prostate cancer risk because of observations in studies of migrant populations [2–4]. World-wide, prostate cancer incidence and mortality rates vary greatly. For example, the difference in risk between Japanese men, a typical low-risk population, and Afro-American men, who have the highest prostate cancer risk in the world, is more than 25-fold. Japanese migrants to the USA have acquired a risk that approaches the risk among US men. These observations suggest that the change of environment, particularly dietary habits, in Japanese migrants has caused this more than 10-fold change in risk, whereas endogenous factors are probably not major determinants of prostate cancer risk.

Epidemiologic research on human prostate carcinogenesis is difficult because of (1) the old age of most prostate cancer patients, (2) the probably very long latent period of this cancer, and (3) the apparent complexity of its etiology. Therefore, several model systems have been developed during the past decade as a surrogate for human prostatic carcinogenesis, all with the rat as experimental animal. These rat models will be discussed here with emphasis on their characteristics in relation to prostate cancer and

Table I. Requirements for Appropriate Animal Models of Prostatic Carcinogenesis

Histology:	Adenocarcinoma
Biologic behavior:	Invasive and metastasizing, preferably to bone; Androgen-sensitive, responding to hormonal therapy, ultimately relapsing to hormone-insensitive state; Slow growing, but not so slowly as to preclude feasible experiments
Embryology:	In rodents, not developing from the ventral prostate, but from dorsal, lateral, and/or anterior prostate lobes
Natural history:	Mimic pathogenesis of human prostatic cancer, with identifiable precursor lesions and a "latent" carcinoma stage
Genetic alterations:	Similar molecular/genetic alterations to those found in human prostatic carcinomas
Feasibility:	Simple yet relevant induction procedure; Predictable and adjustable incidence

prostatic carcinogenesis in humans and their suitability for studies of modification of prostatic carcinogenesis, including chemoprevention.

REQUIREMENTS FOR APPROPRIATE ANIMAL MODELS OF PROSTATIC CARCINOGENESIS

Appropriate and valid animal models for prostatic carcinogenesis must be similar to human prostate carcinomas in their histology, biochemical properties, molecular and genetic characteristics, embryological origin, natural history, and biological behavior, and they must be practical. The most important characteristics required for an appropriate prostatic carcinogenesis animal model are summarized in Table I. Although most animal models do not possess all these characteristics, the more requirements listed in Table I that are met by a model, the more adequate it will be.

The rodent prostate consists of four paired lobes that, unlike in man, have not merged into one anatomic structure [5]. Three of these lobes, dorsal, lateral, and anterior, are homologous to zones in the human prostate. (The rat anterior lobe is more commonly referred to as coagulating gland.) One rat lobe, the ventral prostate, does not appear to have a human homologue [5]. This is important because some animal models involve tumor development selectively in the ventral lobe, which markedly lessens their adequacy.

The tumors developing in rat prostate cancer models need to be androgen-sensitive adenocarcinomas that metastasize, preferably to bone, to represent the disease in man. Although the androgen sensitivity of the available prostate cancer animal models has not been established, those models that require long-term testosterone administration to achieve a high tumor incidence are likely to be androgen-sensitive, as discussed later. Bony prostate cancer metastases seem to be specific for humans, since they have not been documented in spontaneous or induction models of prostatic cancer in rats or in aging dogs; the dog is the only domestic species that develops prostate cancer spontaneously [6]. Information about molecular and genetic alterations in human and experimental prostatic carcinomas is still scarce and only currently emerging [7–13].

Human prostate cancer is a very slow growing tumor that does not occur with appreciable incidence until the sixth decade of life. It is, therefore, desirable that animal models of

prostate carcinogenesis have a relatively long latent period and slow growth rate. However, carcinoma latency in rodent models greatly exceeding 9–12 months is not practical. Similarity of the pathogenesis of prostatic carcinoma in animal models to the natural history of human prostatic cancer is essential. This pathogenesis is reviewed below.

THE PATHOGENESIS OF HUMAN PROSTATIC CANCER

Early studies on the pathogenesis of human prostate cancer have provided evidence for a multi-step process initially involving the formation of microscopic carcinomas which are *not* clinically evident. The prevalence of these so-called latent carcinomas is high in autopsy series of aged men [3,4,14]. Some of these carcinomas progress to clinically evident, metastasizing cancer. Populations that differ in risk for clinically evident prostate cancer have a comparable prevalence of these latent prostate cancers, particularly those cancers that are small and histologically not very invasive [3,4]. The international variation of the prevalence of latent carcinomas that are larger and more invasive somewhat parallels the geographic differences in incidence of clinically evident prostate cancer [2–4]. It has been suggested that non-invasive latent prostate cancer and invasive prostate cancer are different diseases, but currently they are considered different stages of the same process [14,15]. It is likely that the environmental factors that are responsible for the large geographic variation in prostate cancer occurrence enhance progression from small, non-invasive latent carcinoma to larger, invasive cancer [2–4].

A recently recognized premalignant multicentric lesion, duct-acinar dysplasia [16], also known as prostatic intraepithelial neoplasia (PIN) [17], occurs with high frequency in prostates with a carcinoma [16,18,19]. The high-grade stage of this lesion in particular can be continuous with frank carcinoma, which strongly suggests that it can be a direct precursor of carcinoma [16,17,19]. However, as discussed later, it is highly unlikely that all PINs will ultimately progress to carcinoma or that all prostate carcinomas originate from PIN.

A strong correlation between tumor volume and histological (Gleason) grade has been found in some studies [14,20], and McNeal, who has conducted pioneering work on the natural history of prostatic cancer [15,16,19], has proposed a slow, linear growth for human prostatic cancer [20]. However, others have not found as consistent or as strong a correlation between grade and tumor volume [18,21–23]. Furthermore, small, high-grade carcinomas and large, low-grade carcinomas do occur [18,22,24]. Prostate carcinoma was until recently thought to arise predominantly from the peripheral zone of the gland, but rarely from its center [3,20]. New data, however, indicate that a third region in the human prostate, the transition zone [25], also frequently harbors carcinomas, which often are of lower grade initially than those in the peripheral zone [18,25]. High-grade PIN is very often associated with peripheral zone cancers, but not as frequently with these transition zone carcinomas [18,26,27], suggesting that PIN is more likely to be a precursor of peripheral zone cancers than of those originating in the transition zone.

We have recently summarized these data in a multistage model of human prostatic carcinogenesis (Fig. 1) [28]. In this model, clinically evident prostatic cancer, of any clinical stage, can develop via a variety of pathogenetic pathways. These pathways may or may not involve a stage of PIN, and may or may not involve progression from low- to high-grade (some cancers are high-grade early on, whereas other tumors remain low-grade). Growth of the tumor always occurs. This model is an overt simplification in that it does not take into account that carcinoma of the prostate is frequently multicentric, often with a large variation in histological grade within one prostate [4,14,18,25,28], and that different pathways probably predominate in the peripheral or transition zone of the human prostate gland. On the other hand, the model may explain several unresolved issues in human prostate carcinogenesis. Different pathways may be associated with several different etiologies. This could in part explain why prostate cancer epidemiology is so complex and why no major causes of prostate cancer have been identified thus far [2]. Furthermore, the considerable variation among prostate carcinomas in histomorphology and biological behavior may be

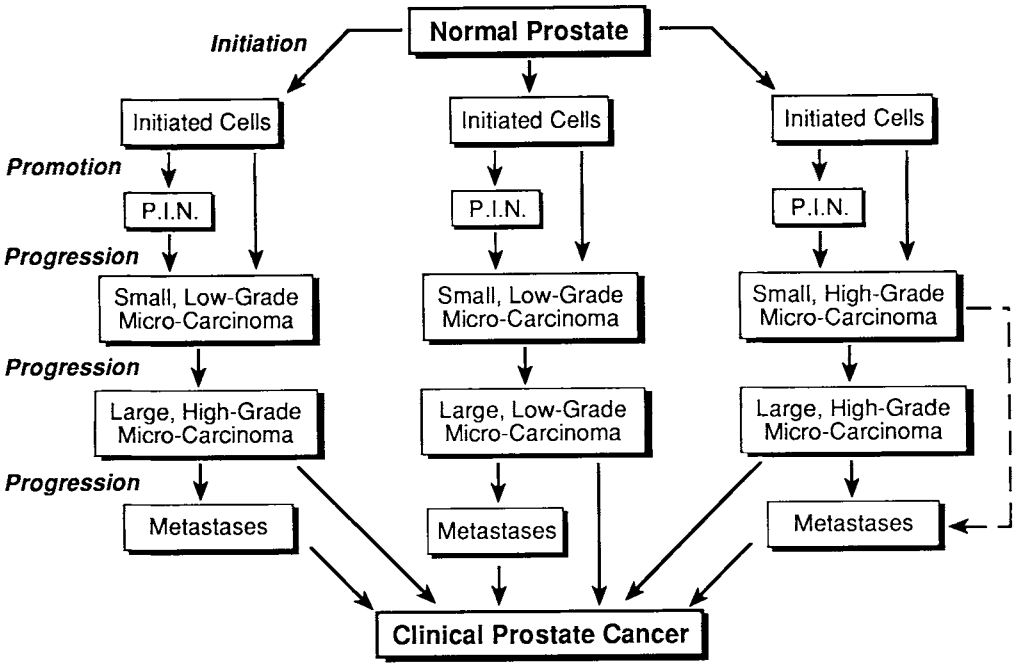

Fig. 1. A working hypothesis of human prostatic carcinogenesis: Multiple pathogenetic pathways including an initiation step, a promotion stage, and several steps in the progression towards clinically evident prostate cancer. Formation of microscopic cancer may or may not involve a stage of PIN development, and development of clinical prostate cancer may or may not be preceded by formation of distant metastases. The interrupted line indicates that it is imaginable that high-grade carcinomas may metastasize even if they are still small. (Adapted from reference 28.)

due to different pathways leading to carcinomas that differ in grade and other characteristics. This multiple-pathway model is also consistent with a previously proposed two-disease model of prostate cancer [2].

If the concept of multiple pathogenetic pathways in human prostatic carcinogenesis is accepted, multiple model systems of prostatic carcinogenesis representing these various pathways need to be developed. Below is a summary of the various prostatic carcinogenesis model systems available to date.

SPONTANEOUS AND INDUCTION MODELS OF PROSTATIC CARCINOGENESIS

Table II summarizes pertinent information about currently available spontaneous and induction models of prostatic carcinogenesis which utilize rats. Those models that satisfy most requirements listed earlier in Table I are italicized in Table II. Neither of the spontaneous models [29,30] is adequate or practical. Induction models of prostate carcinogenesis can be sub-classified as indicated in Table II. Models based on prostate-selective carcinogen metabolism [31,32] have in common that they probably depend on DNA damage in the ventral prostate due to ventral lobe-selective metabolism of the carcinogens used, 3,2′-dimethyl-4-aminobiphenyl (DMAB) [33] and *N*-nitrosobis(2-oxopropyl)-amine (BOP). However, the neoplasms induced are not comparable to those found in men [31,32,34]. Combining hormonal stimulation of prostatic cell proliferation and repeated administration of DMAB markedly increases carcinoma incidence but does not influence tumor type or site of origin [34].

More relevant are prostate carcinomas induced in rats by a single exposure to a variety of chemical carcinogens, such as *N*-methyl-*N*-nitrosourea (MNU), provided that prostatic cell proliferation is stimulated by hormonal manipulation during administration [35]. These adeno-

Table II. Spontaneous and Induction Models of Prostatic Carcinogenesis*

A. Spontaneous models

1. ACI rat: High incidence of cribriform, non-metastasizing adenocarcinoma in ventral prostate of 2 year old rats [29]
2. Lobund-Wistar rat: Metastasizing adenocarcinoma in dorsolateral prostate of aged germfree rats [30]

B. Induction models based on prostate-selective metabolism of chemical carcinogens

1. Long-term treatment with 3,2′-dimethyl-4-aminobiphenyl (DMAB): Non-invasive, intra-acinar, cribriform adenocarcinomas in ventral lobe (F344 rat) [31]
2. Long-term treatment with *N*-nitroso(2-oxopropyl)amine (BOP): Squamous cell carcinomas in ventral prostate (MRC rat) [32]

C. Induction models based on stimulation of prostatic cell proliferation during carcinogen exposure

1. *Sequential treatment with cyproterone acetate and testosterone propionate followed by a single carcinogen injection* [*N*-nitroso-*N*-methylurea (MNU), 7,12-dimethylbenz[*a*]anthracene, or DMAB]: *Low incidence* (5–25%) of adenocarcinomas in dorsolateral and anterior prostate (WU rat) [35,36]
2. Cycles of sequential treatment with ethinyl estradiol (with or without subsequent methyl testosterone) and a single carcinogen injection (DMAB): High incidence (up to 85%) of non-invasive, intra-acinar, cribriform adenocarcinomas in ventral lobe (F344 rat) [34]
3. Short-term testosterone treatment followed by short-term carcinogen administration (DMAB or BOP): very low incidence ($\leq$5%) of prostate carcinoma (F344 or MRC rat) [40,44]

D. Induction models based on androgen action or combined androgen-estrogen action

1. *Chronic treatment with testosterone: Low incidence* (5–20%) of adenocarcinoma in the dorsolateral and/or anterior prostate (various rat strains) [38–43]
2. *Chronic treatment with a combination of testosterone and estrogen (estradiol-17β): High incidence* of adenocarcinoma in the dorsal, lateral, and anterior prostate (NBL or Sprague Dawley rat) [45,46]

E. Induction models based on a combination of chemical carcinogen treatment and hormone action

1. *Single carcinogen injection (MNU) followed by chronic administration of high doses of testosterone: Variable incidence* of adenocarcinomas in dorsolateral prostate (Lobund-Wistar rat) [42,43]
2. Short-term sequential treatment with testosterone and carcinogen (BOP) followed by administration of high doses of testosterone: High incidence of adeno- and squamous cell carcinomas in dorsolateral and ventral prostate (MRC rat) [40]
3. *Chronic administration of high doses of testosterone combined for 20 weeks with intermittent carcinogen injections (DMAB): High incidence* of adenocarcinomas in dorsolateral and anterior prostate (F344 rat) [44]
4. *Sequential treatment with cyproterone acetate and testosterone propionate and a single carcinogen injection (MNU) followed by chronic administration of low doses of testosterone: High incidence* of adenocarcinomas in dorsolateral and anterior prostate (WU rat) [41]

*Those models that satisfy most requirements listed in Table I are italicized, as well as their prostate carcinoma incidence.

carcinomas originate in the dorsolateral or anterior prostate lobes and share some important characteristics with human prostate carcinomas: (1) metastases via the hematogenic and lymphatic routes; (2) considerable variation in degree of histologic differentiation; (3) elevation of serum acid phosphatase; and (4) a long latent period [36]. Early microscopic carcinomas and putative preneoplastic lesions have been identified in this system [36,37]. However, bony metastases have not been described, androgen sensitivity of these carcinomas is uncertain at present, and the incidence is only 5–15% after one year. Spontaneous prostate cancer incidence in most rat strains is between 0.3 and 0.05% at 2.5–3 years of age [37]. Similar carcinomas originating from the same prostate lobes are induced at low incidence by chronic administration of testosterone [38–43]. Both low incidence models, the MNU model and the chronic testosterone administration model, seem suitable for studying enhancement of prostate carcinogenesis by dietary factors, for example.

HIGH INCIDENCE ANIMAL MODELS OF PROSTATIC CARCINOGENESIS

A high incidence of adenocarcinomas is required to study inhibition and chemoprevention of prostatic carcinogenesis. Such a high incidence can only be achieved by treating rats with a combination of carcinogens and testosterone, or testosterone and estrogens. The high incidence models listed in Table II are compared in more detail in Table III. MNU injected without concomitant stimulation of cell proliferation appears to result in a variable incidence of accessory sex gland carcinomas in Lobund-Wistar rats when MNU is followed with chronic testosterone treatment [42,43]. This variable tumor yield from experiments using the exact same protocol suggests that there are genetic variants of the Lobund-Wistar strain that differ in susceptibility to accessory sex gland carcinoma induction. The studies with BOP plus chronic testosterone treatment in Wistar-derived MRC rats demonstrate that stimulation of cell proliferation at the time of carcinogen injection is highly effective in enhancing the yield of accessory sex gland carcinomas [40]. However, since this BOP system involves production of a significant number of squamous cell carcinomas and tumors in the ventral prostate [40], it is not a very adequate animal model (see Table I).

The combination of stimulated cell proliferation at the time of carcinogen injection to rats (a single dose of MNU injection or repeated DMAB administration) and subsequent chronic testosterone treatment provides the most adequate high incidence animal model of prostatic carcinogenesis presently available [41,44]. The tumor incidence (75–85%) is adequate for chemoprevention studies, and seems not to be very dependent on rat strain (both inbred F344 and outbred Wistar WU rats are responsive) or carcinogen [41,44]. The tumors induced are adenocarcinomas with the same characteristics as those induced in the low incidence MNU and the chronic testosterone administration models described earlier [36]; those induced by MNU plus chronic testosterone administration in the Lobund-Wistar rat [42,43]; and some of the carcinomas induced by BOP plus chronic testosterone administration [40].

Chronic administration of testosterone plus estradiol-17β produces adenocarcinomas in the prostate [38,45] of 100% of inbred NBL rats, and 20–30% of Sprague Dawley rats [46]. These carcinomas are all well-differentiated and appear to arise exclusively from the periurethral portion of the ducts of the dorsolateral and anterior prostates [46]. In contrast, carcinomas induced by MNU, DMAB, and/or chronic testosterone administration vary considerably in degree of histologic differentiation and are never found associated with the proximal, periurethral portion of dorsolateral and anterior prostate ducts. Thus, carcinomas produced by chronic testosterone plus estradiol-17β administration probably represent an animal model of a different pathogenetic pathway of prostatic carcinogenesis than those induced by MNU, DMAB, and/or chronic testosterone. However, this model is at present not suitable for chemoprevention studies because (1) early death of the rats from estrogen-induced pituitary tumors seems to prevent the carcinomas from growing to a macroscopic size and metastasizing, and (2) the incidence is 100%, which is undesirably high for a animal model in cancer chemoprevention studies.

Table III. High Incidence Induction Models of Prostatic Carcinogenesis[1]

Reference	Strain	N	Carcinogen	Cell Proliferation[2]	All Carcinomas[3]	Macroscopic Size[3]	Microscopic Size[3]
42	Lobund Wistar	40	MNU[4]	–	36 (90)	31 (77)	5 (13)
43	Lobund Wistar	41	MNU[4]	–	10 (24)	9 (22)	1 (2)
40	Wistar MRC	23	BOP[4]	–	4 (17)[5]	4 (17)	0
		106	BOP[4]	+	70 (66)[6]	50 (47)	20 (19)
44	Fischer F344	37	DMAB[4]	+	28 (76)	14 (38)	14 (38)
41	Wistar WU	60	MNU[4]	+	50 (83)	27 (45)	23 (38)
45	NBL	12	Testosterone + Estradiol-17β	N/A	12 (100)	0	12 (100)

[1] Data are based as much as possible on the incidence of carcinomas in the dorsolateral and/or anterior prostate, but not the ventral prostate or seminal vesicle, reported in the listed references.
[2] The presence (+) or absence (–) of stimulation of prostatic cell proliferation during carcinogen treatment.
[3] Number of animals with prostate carcinomas; in parentheses the percentage of animals with carcinomas.
[4] Carcinogen treatment was followed by chronic testosterone administration.
[5] Three of these tumors (13%) were squamous cell carcinomas and/or they were located in the ventral prostate.
[6] Twenty-eight of these tumors (26%) were squamous cell carcinomas and/or were located in the ventral prostate.

Approximately 50% of the carcinomas induced in F344 or WU rats by MNU or DMAB after stimulation of cell proliferation followed by chronic testosterone treatment are of microscopic size [36,41,44]. This is an important feature for two reasons: First, the exact site of origin of macroscopic-size carcinomas is often unclear and could be the seminal vesicle, a very infrequent site of tumor development in men. The presence of microscopic-size carcinomas that are clearly localized in the dorsolateral or anterior prostate lobes strongly suggests that a significant proportion of the larger carcinomas also originated from these lobes. Most carcinomas in the Lobund-Wistar and BOP models are macroscopic in size (Table III) [40,42,43] and therefore of uncertain origin. Second, shifts from macroscopic to microscopic carcinomas or *vice versa*, and from microscopic carcinomas to earlier stages of tumor development, such as atypical hyperplasia or *vice versa*, can indicate effects on tumor progression of chemopreventive agents, for example.

The following approach is a practical method to assess tumor progression in rat models of prostatic carcinogenesis. Using criteria proposed elsewhere [36,37], first classify proliferative lesions in the dorsal, lateral, and/or anterior prostate as: (1) atypical hyperplasia, (2) carcinoma *in situ* (more than just atypical hyperplasia, but no clear invasion), (3) microscopic-size carcinoma (clear invasion, but not macroscopi-

cally visible), (4) macroscopic carcinoma without metastases, or (5) macroscopic carcinoma with (pulmonary) metastases. (Categories 4 and 5 almost always involve more than one structure, but can be sub-classified according to the structures they involve.) Subsequently, compare the distribution of these types of lesions among experimental groups, and determine whether there are shifts in this distribution that are associated with the modifying (chemopreventive) treatment.

MECHANISMS OF PROSTATIC CARCINOGENESIS IN ANIMAL MODELS

Ideally, animal models of prostatic carcinogenesis are not only similar to human prostatic cancer with regard to pathogenesis, but also involve similar carcinogenic mechanisms. A causal relation between androgens and prostate carcinoma development in man and animal models is biologically very plausible [2,47]. Chronic treatment with testosterone is a weak complete carcinogen and a strong tumor promoter for the rat prostate, and chemical carcinogens such as MNU and testosterone act synergistically in producing prostate carcinomas, as indicated by the earlier-mentioned studies [38–44]. Male human populations that are at high risk for prostate cancer often, but not always, have slightly higher circulating testosterone levels than populations that have a lower risk [2,47,48]. Nevertheless, the mechanism of the carcinogenic and tumor-promoting effects of testosterone on the rat prostate remains unknown, although it is likely that androgens act via a receptor-mediated mechanism [28].

Activation of *ras* proto-oncogenes is frequently involved in human and experimental tumors [49,50]. We examined prostate carcinomas, induced in rats by MNU and long-term testosterone treatment, for the presence of activating *ras* gene point mutations using polymerase chain reaction techniques, and found a $G^{35} \rightarrow A$ mutation in codon 12 of the K-*ras* gene in 7/10 prostate carcinomas [7]. No mutations were detected at other positions of the K-*ras* gene or in the H-*ras* gene. The molecular mechanism of one of the first steps of the multistage process of MNU-induced rat prostatic carcinogenesis is most likely the production of O^6-methylguanine adducts in the K-*ras* 12th codon by MNU, followed by base mispairing during replicative DNA synthesis [7]. While the mechanism responsible for the earlier-mentioned synergism between MNU and testosterone in producing rat prostate carcinomas remains unclear, we postulate that prostatic epithelial cells with the activated oncogene have a selective growth advantage over other cells in the presence of excess androgens [7]. Activation of K-*ras* and H-*ras* genes has been reported in 4% to 26% of human prostate carcinomas [8–11].

The mechanism of prostatic carcinoma induction by estradiol-17β and testosterone is not known. Induction of the type II estrogen receptor in the dorsolateral prostate may be involved [51]. Preliminary observations [46] suggest that induction by estradiol of DNA adducts as detected by ^{32}P-postlabeling may play a role. While it is unknown whether testosterone is a tumor promoter in this system, our preliminary findings suggest that estradiol-17β acts as a tumor initiating agent.

CONCLUSIONS

1. Several animal model systems of prostatic carcinogenesis are available, both low incidence models for studying enhancement and high incidence models for studying inhibition of carcinogenesis.
2. Models based on combining carcinogen treatment (MNU or DMAB injection while prostatic cell proliferation is stimulated) with chronic testosterone administration are presently the most adequate high incidence models, and thus the most suitable for chemoprevention studies.
3. Other high incidence models, particularly prostate cancer induction by chronic treatment with estradiol-17β and testosterone, must be further refined before they are suitable for chemoprevention studies. More research is also needed into the mechanisms of both experimental and human prostatic carcinogenesis.

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