

## The Prostate: A Target for Carcinogenicity of 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) Derived from Cooked Foods<sup>1</sup>

Tomoyuki Shirai,<sup>2</sup> Masashi Sano, Seiko Tamano, Satoru Takahashi, Masao Hirose, Mitsuru Futakuchi, Ryohei Hasegawa, Katsumi Imaida, Ken-ichiro Matsumoto, Keiji Wakabayashi, Takashi Sugimura, and Nobuyuki Ito

First Department of Pathology, Nagoya City University Medical School, 1-Kawasumi, Muzuho-cho, Mizuho-ku, Nagoya 467, Japan [T. Sh., M. S., M. F., S. Tak., M. H., R. H., K. I., N. I.]; Daiyu-kai Institute of Medical Science, Nishiazai, Ichinomiya, Aichi-Prefecture 491-01, Japan [S. Tam.]; and Biochemistry Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Tokyo 104, Japan [K. M., K. W., T. Su.]

### Abstract

Prostate tissues obtained from rats given a food-derived carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), at a dose of 400 ppm in the diet for 52 weeks were histopathologically evaluated and found to contain prostate carcinomas limited to the ventral lobe in 18 of 27 cases. Atypical hyperplasias were also detected in the ventral and anterior prostate as well as the seminal vesicles. <sup>32</sup>P-Postlabeling analysis of DNA demonstrated that PhIP-DNA adducts are produced in all lobes of the prostate of rats receiving PhIP. The findings indicate that PhIP is carcinogenic to rat prostate in addition to the previously demonstrated targeting of the colon and mammary glands, providing evidence of a possible role of PhIP in human prostate carcinogenesis and highlighting the potential importance of PhIP for man.

### Introduction

Most human cancers are believed to be caused by the exposure of individuals to environmental carcinogenic agents, with diet playing a particularly important role (1-3). In recent years, a number of HCA<sup>3</sup> pyrolysis products have been isolated from cooked fish and meat and have been revealed to be highly mutagenic in the Ames test (2, 4, 5) as well as carcinogenic to rodents (4). One of the most prevalent of these carcinogenic HCAs, PhIP, was found to exert carcinogenicity in the mammary glands of female F344 rats and the colon of males (6). Because both organs are common sites of cancer development in Western countries where people consume large quantities of meat, PhIP has attracted special attention as a human cancer risk factor. The carcinogenicity of PhIP in the colon and mammary glands of rats was confirmed by subsequent experiments using Sprague Dawley rats (7) and different doses (8).

It has been demonstrated in our laboratory that DMAB induces carcinomas in the ventral prostate, mammary glands, colon, and urinary bladder (9-11). DMAB has a structure somewhat similar to carcinogenic HCAs, and this prompted us to investigate whether PhIP might also induce prostate carcinomas in rats.

### Materials and Methods

Prostate tissues were obtained from our previously published experiment (6) in which a total of 70 male F344 rats (Charles River Japan, Inc., Atsugi, Japan)

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<sup>2</sup> To whom requests for reprints should be addressed.

<sup>3</sup> The abbreviations used are: HCA, heterocyclic amine; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; DMAB, 3,2'-dimethyl-4-aminobiphenyl; BrdUrd, bromodeoxyuridine.

were divided into treated (30 animals) and control (40 animals) groups. The former were given PhIP mixed in the diet (Oriental MF powdered diet; Oriental Yeast Co., Tokyo, Japan) at a dose of 400 ppm for 52 weeks. PhIP hydrochloride was synthesized in the NARD Institute (Osaka, Japan), and its purity was >99.9%. Procedures involving animals and their care were conducted in conformity with institutional guidelines in compliance with national and international law and policies. All surviving rats were killed by exsanguination from the aorta under light ether anesthesia at the end of week 52. Animals that died earlier or were killed in a moribund state were also autopsied. The prostates, including the seminal vesicles, were fixed in 10% buffered formalin. For tissue preparation of the accessory sex organs, single sagittal slices through the ventral prostate and the dorsolateral prostate including the urethra and one transverse sample from each side of the seminal vesicles including the anterior prostate were embedded in paraffin. Sections (4 mm) through all tissues were cut and stained with H&E for histological examination.

Additional experiments were carried out to examine DNA adduct formation and cell proliferative activity. Eight 6-week-old male F344 rats were given a diet containing 400 ppm PhIP for 4 weeks and then killed. Five of them received a single i.p. injection of BrdUrd (20 mg/kg body weight) 2 h before sacrifice, when the accessory sex organs, liver, kidney, and colon were removed for histological examination. The prostates and livers of the remaining three rats that were not administered BrdUrd were frozen in liquid nitrogen for DNA extraction and stored at -80°C until use. The incorporation of BrdUrd into the nuclei was visualized immunohistochemically using the avidin-biotin-peroxidase complex method with a monoclonal antibody against BrdUrd. Labeling indices were generated by counting the number of labeled cells among at least 2000 epithelial cells/region and expressed as percentage values.

The difference in the incidence of prostate lesions was analyzed using Fisher's exact probability test, and the difference in BrdUrd labeling indices was analyzed using Student's *t* test.

### Results

The observed histopathological lesions of the accessory sex organs were classified as described previously (9, 10, 12) into atypical hyperplasia and carcinoma categories (Fig. 1, A-E). The incidences of atypical hyperplasias in the ventral prostate and seminal vesicles (Fig. 1F) as well as those of carcinomas in the ventral lobe were significantly increased in the group receiving 400 ppm PhIP (Table 1). A tendency for increase was also observed for atypical hyperplasias in the anterior lobe of the prostate. All lesions were histopathologically comparable with those induced by DMAB (10, 11, 13) and were sometimes found multifocally. The carcinomas were all microscopic in size, demonstrating cribriform microglandular patterns (Fig. 1, C-E) and frequently involving adjacent acini with infiltrative or invasive growth.

Four weeks of treatment with 400 ppm PhIP significantly increased BrdUrd uptake in the ventral and dorsolateral lobes of the prostate and caused a slight elevation in the anterior lobe and seminal vesicles (Table 2), suggesting that PhIP increases DNA synthesis in epithelial cells throughout the accessory sex organs. PhIP-DNA adduct formation was analyzed in the ventral, dorsolateral, and anterior lobes of the

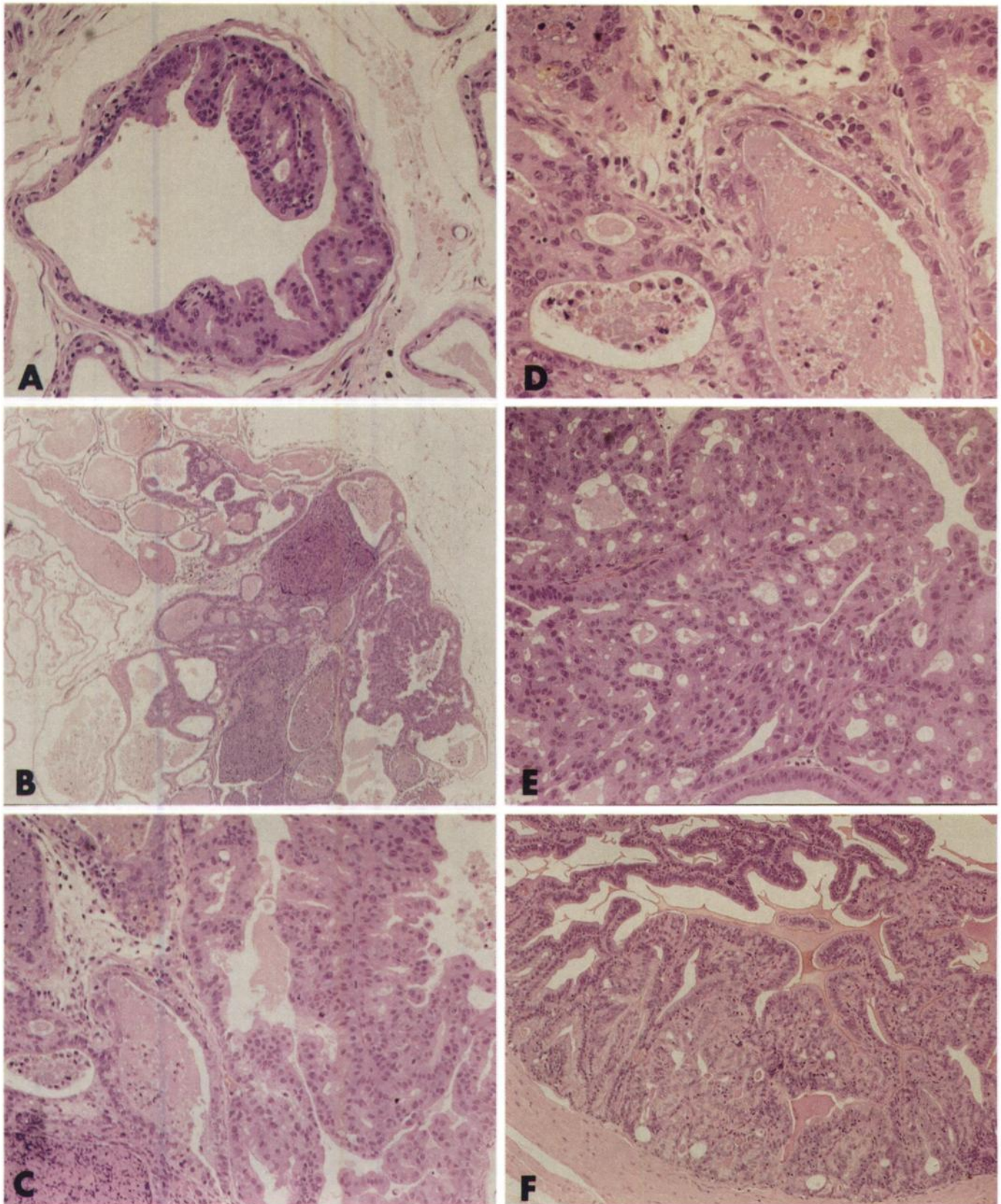


Fig. 1. Histopathological appearance of prostate and seminal vesicle lesions in rats given 400 ppm PhIP for 52 weeks. All photographs were taken of H&E-stained specimens. *A*, atypical hyperplasia of the ventral prostate. Note the replacement of the lining epithelial cells with dysplastic proliferating cells ( $\times 200$ ). *B*, carcinoma of the ventral prostate forming a relatively large mass ( $\times 40$ ). *C*, higher magnification of *B*. Atypical tumor cells demonstrate papillary or glandular patterns ( $\times 200$ ). *D*, cellular and structural atypia in the carcinoma illustrated in *B* ( $\times 400$ ). *E*, another example of a ventral carcinoma showing a predominantly cribriform pattern ( $\times 400$ ). *F*, atypical hyperplasia in a seminal vesicle. The lining epithelial cells are partly replaced with dysplastic proliferating cells ( $\times 100$ ).

Table 1 Incidences of prostate lesions in rats given dietary 400 ppm PhIP for up to 52 weeks

Group	Effective no. of rats	No. of rats with lesions in the <sup>a</sup>				
		Ventral prostate		Anterior prostate		Seminal vesicles
		Atypical hyperplasia	Carcinoma	Atypical hyperplasia	Atypical hyperplasia	
PhIP (400 ppm)	27	22 (81.5) <sup>b</sup>	18 (66.7) <sup>b</sup>	8 (29.6)	24 (88.9) <sup>b</sup>	
Control	37	1 (2.7)	0 (-)	0 (-)	2 (5.4)	

<sup>a</sup> Numbers in parentheses are percentage values.

<sup>b</sup>  $P < 0.001$ .

prostate and the seminal vesicles of rats fed 400 ppm PhIP under modified adduct intensification conditions (14, 15). A single adduct spot corresponding to authentic *N*-(deoxyguanosin-8-yl)-PhIP 5'-monophosphate was detected in all sites investigated, with levels of  $8.93 \pm 2.56$ ,  $8.19 \pm 2.51$ , and  $4.29 \pm 1.69$  per  $10^7$  nucleotides in the ventral, dorsolateral, and anterior lobes of the prostate, respectively, and a level of  $3.99 \pm 0.57/10^7$  nucleotides in the seminal vesicles (Table 3). DNA samples from the colon, pancreas, and liver were found to contain adduct levels of  $4.85 \pm 0.82$ ,  $13.1 \pm 4.29$ , and  $0.57 \pm 0.19$  per  $10^7$  nucleotides, respectively.

## Discussion

The initial discovery of PhIP carcinogenicity in the rat colon and mammary glands (6) attracted much attention because of the fact that PhIP is relatively abundant in cooked meat and fish, and man may therefore be exposed to appreciable amounts on a daily basis (16). Moreover, mammary and colon carcinomas are both common malignancies in Western countries, along with prostate lesions (17). Although it is not rare for spontaneously developed ventral prostatic proliferative lesions to be encountered in old male F344 rats in 2-year bioassay experiments (18, 19), they are extremely infrequent in such animals at the age of about 1 year (9, 20). Thus, the finding of high incidences of lesions and even carcinomas of the ventral prostate in the present study is exceedingly significant. The unequivocal association with administration of PhIP provides strong evidence that this HCA may play a role in development of human prostate cancer, the most common type of malignancy in American men (17, 21). In our previous 2-year study with lower doses of PhIP (100 and 25 ppm in the diet), no increase in the incidence of ventral prostate carcinomas comparable to that seen in the present experiment was observed (8). This suggests that relatively high doses are necessary for carcinogenicity to be exerted in the prostate, unlike the mammary gland and colon cases.

PhIP has previously been shown to modify DNA in various organs of rats (22). Based on the data obtained in the present and previous studies, the prostate is clearly a major target of PhIP-DNA adduct formation in the rat. DMAB also forms DNA adducts in the rat prostate (23) and induces ventral carcinomas. In particular, invasive and metastasizing prostate and seminal vesicle tumors are found when carcinogen treatment is combined with testosterone administration (24, 25). There is thus the possibility that PhIP could also induce frankly malignant prostate carcinomas under similar conditions.

Table 2 BrdUrd incorporation into epithelial cells of the accessory sex organs<sup>a</sup>

BrdUrd was injected 2 h before killing rats fed 400 ppm PhIP for 4 weeks.

Group	Prostate				Seminal vesicles
	Ventral	Dorsolateral	Anterior		
PhIP (400 ppm)	$0.94 \pm 0.05^b$	$1.90 \pm 0.50^c$	$2.21 \pm 0.65$		$4.72 \pm 1.99$
Control	$0.46 \pm 0.20$	$1.06 \pm 0.38$	$1.58 \pm 1.29$		$2.39 \pm 1.71$

<sup>a</sup> Data represent mean percentage  $\pm$  SD from five animals.

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup>  $P < 0.01$  as compared to the contralateral values.

Table 3 PhIP-DNA adduct formation in rat tissues after a 4-week diet of 400 ppm PhIP

The <sup>32</sup>P-postlabeling assay was applied for detection of DNA adducts under modified intensification conditions.

Tissue	DNA adducts (no./10 <sup>7</sup> nucleotides)
Prostate	
Ventral lobe	$8.93 \pm 2.56$
Dorsolateral lobe	$8.19 \pm 2.51$
Anterior lobe	$4.29 \pm 1.69$
Seminal vesicles	$3.99 \pm 0.57$
Colon	$4.85 \pm 0.82$
Pancreas	$13.11 \pm 4.29$
Liver	$0.57 \pm 0.19$

The potential risk of PhIP as a prostate carcinogen or promoter in man as well as the question of whether other carcinogenic HCAs target the prostate clearly warrants further evaluation in the immediate future. As intimated in our previous work, in which maternal exposure to PhIP was found to increase mammary carcinoma development (14), transplacental and trans-breast milk exposure might also be an important factor requiring attention in consideration of possible preventive measures.

Whatever the route, the demonstrated carcinogenicity of PhIP in the colon, mammary, and prostate strongly suggests that minimizing the consumption of well-cooked meat and fish is to be recommended as a preventive strategy.

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