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The Lobund-Wistar Rat Model of Prostate Cancer

Morris Pollard, Ph.D.

Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556

Abstract Two models of preventable metastasizing autochthonous prostate adenocarcinoma (PA) have been described in Lobund-Wistar (L-W) rats: spontaneous PAs that develop at a mean age of 26 months; and induced PAs that develop at a mean time of 10.5 months. Both are similar in many respects to the counterpart disease in man.

PAs develop spontaneously, and by induction through a combination of *N*-methyl-*N*-nitrosourea (MNU)/testosterone treatments. Our investigations with L-W rats show that PA is manifested spontaneously in 26% of aged L-W rats, and by induction in approximately 90% of younger rats. It is characterized by metastatic adenocarcinoma initiated in, and expanding from, the dorso-lateral and anterior lobes, and occasionally in the seminal vesicles. It is regulated by genetic, hormonal, and age-related mechanisms. Spontaneous PAs are prevented by life-long moderate (25%) diet restriction and, in rats at risk of developing induced PA, by early treatments with estradiol, with dihydrotestosterone, with a retinoid, and by castration. While the "premalignant" stages of induced tumorigenesis are susceptible to intervention, the overtly malignant stage resists therapeutic trials with the same agents and procedures. The transition from dependency to autonomy has not yet been defined.

Key words: cancer metastasis, oncoprevention, premalignant cancer, prostate cancer

A recent workshop report on prostate cancer, sponsored by the National Cancer Institute (U.S.), emphasized the need for a model system of this disease [1]. Several model systems of autochthonous prostate cancer have been reported in rats [2–5]. Assessments of them reveal that they are of limited use since they are based on animal strains with (a) low disease spontaneity, (b) low incidences of clinically evident, induced tumors, (c) long latency periods, (d) infrequent metastatic spread, and (e) the development of additional neoplasms in tissues other than the prostate gland. The model system that has been developed in Lobund-Wistar (L-W) rats fulfills many of the prerequisites which are absent in the above noted models [6,7].

In the course of examining aged, germfree (GF) L-W rats in 1973, four were observed with a solid neoplasm in the pelvic region which was identified as prostate adenocarcinoma (PA). Three of the rats had developed metastatic tumors predominantly in their lungs [8]. Subsequent examinations increased the total count to 9, 7 of which were metastatic [9]. The L-W rat was derived from the standard Wistar rat that had been propagated, at random, through 56 generations under GF status. While not intentionally inbred, they did not reject reciprocal skin transplants. They were free of detectable microbial flora. The types of spontaneous neoplasms that were detected most frequently among them and among conventionalized derivatives were PAs and hepatic tumors, the latter among rats older than 30 months [10].

Models of prostate cancer in L-W rats were developed along 3 lines involving spontaneous, induced, and transplantable PAs.

1. Spontaneous PA. Among conventional L-W rats fed diet L-485 *ad libitum*, 19/72 (26%) developed large PAs at a mean age of 26 months; 18/19 (95%) had metastases in the lungs [10] (Table I). The average weight of the 19 PAs was 13.52 gm. The size of the PA was the result of expansion of the neoplasm into the entire gland, including seminal vesicles. PAs were palpable in the abdomen as hard masses with knobby surfaces when 0.5 cm in diameter; these eventually enlarged to 3–4 cm in diameter. In contrast, 3/47 (6%) of L-W rats on 25% reduced dietary intake developed the same type

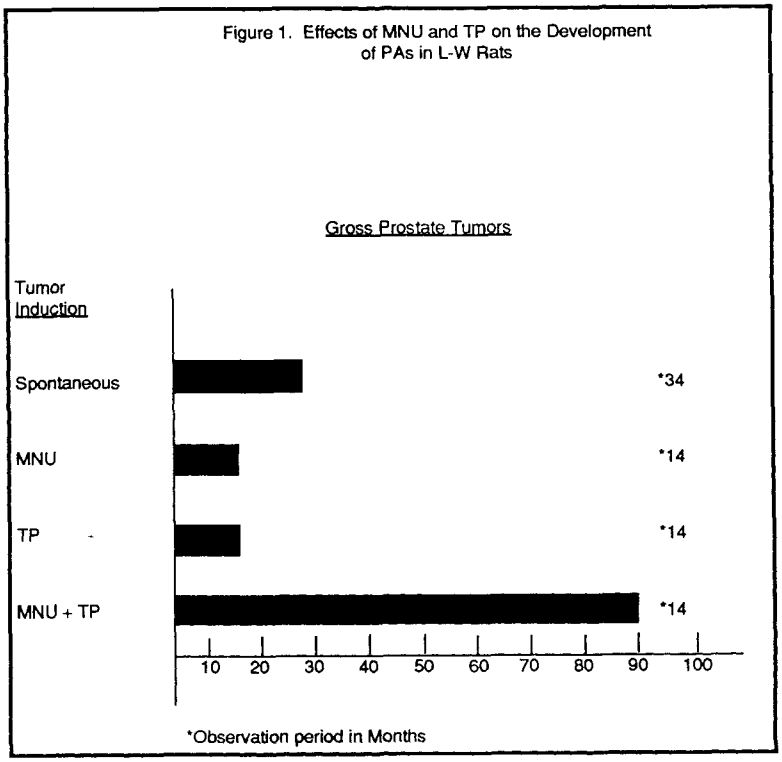
of spontaneous metastasizing PA at a mean age of 36 months [10]. Among disease-free rats, the average weight of the prostate gland was 2.0 gm.

2. Induced PA. Three experiments contributed to the procedure for induction of palpable primary PAs in conventional L-W rats [6,7] (Fig. 1): (a) I.V. inoculation of 3 month old L-W rats with *N*-methyl-*N*-nitrosourea (MNU) (30 mg/kg BW) produced PAs in approximately 10% within the 14 month observation period; (b) four subcutaneous implants of 45–50 mg testosterone propionate (TP) encased in silastic membrane (each at intervals of 2 months) produced PAs in 14% of L-W rats within 14 months; (c) the combination of MNU fol-

Table I. Baseline Data
Prostate Adenocarcinoma (PA) in Lobund-Wistar Rats

	PA Type	Incidence	Average Latency	Average Wt/gm	Metastasis			
					Total	In Lung	In Peritoneum	In Both Sites
I.	Spontaneous	19/72 (26)*	26 months	13.52	18/19 (95)	10/18 (56)	0/18 (0)	8/18 (44)
II.	Induced MNU/TP	167/185 (90)	10.5 months	15.94	131/167 (78)	15/131 (11)	38/131 (29)	78/131 (60)
III.	Transplanted (PA-III)	600/600 (100)**	1 month	12.5	588/600 (98)	588/588 (100)	–	–

* Number positive/number at risk (%)
** Inoculated S.C.



MNU—*N*-methyl-*N*-nitrosourea
TP—Testosterone propionate
PAs—Prostate adenocarcinomas
L-W—Lobund-Wistar

Fig. 1. Effects of MNU and TP on the Development of PAs in L-W Rats

lowed by TP 7 days later induced PAs in 167/185 rats (90%) at a mean time of 10.5 months, and the mean weight of the PAs was 15.94 gm. Seventy-eight percent (131/167) of these PA-bearing rats developed metastases in the lungs and/or the peritoneal cavity (Table I). In about 90% of rats, the PAs involved the entire gland including the ventral lobe, the coagulation gland and the seminal vesicles. The observation period of 14 months was based on our records that no PA developed spontaneously in L-W rats under age 20 months. It is likely that the latency periods for PA development were actually shorter than those reported because the rats were autopsied before the realization that smaller PAs could be detected in the abdomen by palpation. Earlier stages of tumorigenesis involved the dorso-lateral lobe which was palpable as a hard fixed mass with knobby surfaces in the pelvic region. In some instances a "floating" palpable mass actually revealed that the tumor was in the seminal vesicle. PAs in the seminal vesicles were not observed in rats with PAs that developed spontaneously.

The PAs were scirrhotic when incised and were identified as moderately differentiated adenocarcinomas in a prominent matrix of connective tissue. The large tumors were relatively cell-free centrally or liquified, with accumulations of myelocytes dispersed among the tumor cells. Numerous active foci of tumor cells were observed at the peripheral areas of the primary tumor, or disseminated uniformly throughout the metastatic tumors. In about 80% of the rats, the PAs had metastasized through lymphatic channels to the lungs or through the prostate capsule to the peritoneal cavity (Table I).

In the MNU/TP system, MNU served as an initiating agent and TP served as a promoter [11]. The promotional effect of TP was demonstrable when begun as long as 3 months after exposure of L-W rats to MNU, and the incidence of rats with PAs was related to the number of TP implants administered to the MNU-sensitized rats (4 implants were optimal). The identity of initiating agent(s) and promotional agents in rats with spontaneously developed PAs is unknown.

It is fortuitous that the L-W rat is genetically susceptible to PA. In support of this statement, aged GF and conventional Lobund Sprague-Dawley (S-D) rats did not develop PA spontaneously under conditions comparable to those of the L-W rats. S-D rats responded to an I.V. inoculation of MNU with a high incidence of leukemia and/or intestinal cancer (unpublished data). In response to 4 subcutaneous implants of TP, 1 of 25 S-D rats developed a metastasizing PA similar in all respects to the PAs in L-W rats [12]. The MNU/TP system induced a high incidence of leukemia in Hilltop strain Wistar rats (unpublished data).

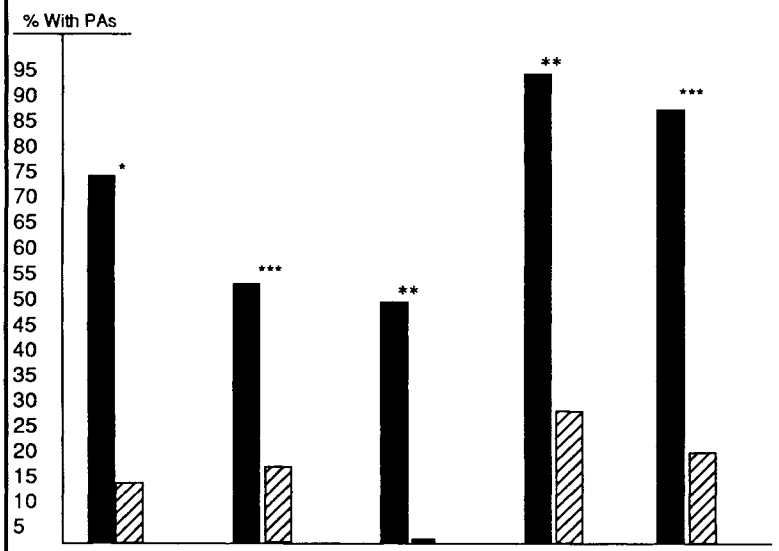
3. Transplanted PA. Six transplantable PA cell lines were derived from spontaneous PAs in aged GF L-W rats. Five of the tumors metastasized uniformly via lymphatic channels from the subcutaneous implant site to the lungs, and a sixth metastasized through blood and lymphatic channels [9].

PREMALIGNANT LESIONS

Induced autochthonous PAs in L-W rats provide a system for investigating tumorigenic mechanisms from initiation to promotion to progression stages. From clinical examination, the first two stages were not apparent and likely premalignant; however, they were the most susceptible to measures aimed at intervention. Examination of MNU/TP-treated rats prior to detection of palpable tumors revealed multifocal hyperplastic changes among the ductal and acinar lining cells in the dorso-lateral lobes of prostates which either protruded into the lumen or expanded into the surrounding connective tissue matrix. The identification of such adenomatous foci as *in situ* lesions was more definitive when mitotic figures were detected among the cells. It is hypothesized that MNU-sensitized prostate cells remained sequestered, dormant and unrecognized for months in L-W rats; however, the addition of testosterone activated the tumorigenic process [11]. Long term treatment of MNU-sensitized L-W rats with implants of estradiol cypionate resulted in involution of epithelial ductal and acinar components in the prostate gland, involution of testes, and swollen pituitary glands. None of the MNU-sensitized estradiol-treated rats developed PA. When the estradiol was replaced by testosterone, the tumorigenic process was activated and produced large metastasizing PAs similar to those noted in Table I (unpublished information).

L-W rats that were treated with the MNU/TP combination were subjected to treatments with several putative chemopreventive drugs or by surgical castration to determine their effects on tumor development and metastasis. The rats were treated at a time midway in the projected latency period for tumor development, *i.e.*, about 5–6.5 months [13] (Fig. 2). Treatments with subcutaneous implants of estradiol or dihydrotestosterone interfered significantly with the development of prostate tumors; a similar interference was observed in rats that had been surgically castrated. Also, administration of 4-hydroxyphenylretinamide (1 mmol) in the diet significantly reduced the incidence of PAs [14]. There were no demonstrable effects on metastasis among those tumors that did develop soon after onset of treatments. The effects of the preventive measures are interpreted as anti-promotional. The transition point between dependency and autonomy has not yet been determined. Administration in the diet of piroxicam, a nonsteroidal anti-inflammatory drug, or of difluoromethylornithine (DFMO), a polyamine inhibitor, did not significantly modify the pattern of growth or spread of the autoch-

Figure 2. Prevention of Induced Prostate Cancer Development in L-W Rats



Rats received an initial I.V. inoculation of MNU (30 mg/kg BW) followed by TP: *-2 implants; **-3 implants; ***-4 implants. Treatments were started at 5-6.5 months. Silastic implants with drugs contained: 30 mg Estradiol; 60 mg DHT; 4-HPR was administered in the diet. The experiment was terminated at 14 months. Black bars = No preventive treatment; Striped bars = Preventative treatment as indicated below bars.

PAs—Prostate adenocarcinomas
DHT—Dihydrotestosterone
4-HPR—4-hydroxyphenylretinamide

Fig. 2. Prevention of Induced Prostate Cancer Development in L-W Rats

thonous PAs. Further assessments may require a reduced level of testosterone treatments. None of the preventive agents and procedures noted above was of therapeutic value.

CONCLUSION

It is of interest to note the high incidence of "dormant" or *in situ* tumor foci in the prostates of men, for example, in China and in Japan, compared with the low incidence of clinical disease among those populations [15]. The incidence of PA in the progeny of Asians rises after they migrate from the Orient to the West [16] which is attributed to "environmental" factors, possibly food. The nature of and the activation of dormant (*in situ*) prostate tumor foci are subjects of high priority to investigators of prostate cancer. Model systems will clarify the mechanisms involved in the activation process.

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