MINIREVIEW

Hormone-Refractory Prostate Cancer in the Lobund-Wistar Rat

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Research on cancer prevention and therapy must focus on the refractory disease, the fatal end-stage of cancer that develops in patients with organ-related solid tumors. Refractory cancers develop spontaneously in advanced-stage tumors or in relapsed cases after failed therapy. Because neither prevention nor therapy is currently feasible, refractory cancer is a major impediment to survival. There is a great need for an animal model of prostate cancer (PC), one that develops cancer from initial premalignant to the terminal refractory stages. We describe here a model of hormone-refractory prostate cancer (HRPC) that develops spontaneously through two stages by endogenous mechanisms in the Lobund-Wistar (LW) rat. The early premalignant, testosterone (T)-dependent stage is promoted by high levels of endogenous T, and up to age 12 months is reversible by T deprivation; without this intervention, the tumorigenic process progresses to the refractory stage, which is highly aggressive and does not respond to T deprivation or to a wide range of therapies. Initial refractory tumors are palpable at approximately 18 months of age. As they continue to grow, the tumors express characteristics seen in refractory cancers in humans (i.e., hypoxia, expression of hypoxia-inducible factors, and metastasis). Chemically induced HRPCs in LW rats manifest the same two developmental stages, but with shorter latency periods. A transplantable, metastasizing cell line (PAIII) was derived from a germfree LW rat with advanced-stage cancer. Both spontaneous and chemically induced autochthonous HRPC model systems serve as outstanding models for studies on the prevention and therapy of refractory cancer. Exp Biol Med 230:520–526, 2005

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Introduction

In the evolution of the neoplastic state, progression moves from dependence to autonomy (1). The latter (refractory) stage, manifested in a wide variety of solid tumors, including hormone-refractory prostate cancer (HRPC), is a major impediment to survival. If untreated, or in the event of relapse after failed therapy, patients develop irreversible, aggressive, therapy-resistant metastatic disease.

It was observed that when rapidly replicating tumor cells propagate beyond their optimal supply of oxygen, cells most distant from the source of oxygen suffer anoxic necrosis (2), and adjacent surviving pimonidazole-stained hypoxic cells (3) manifest changes related to the refractory disease. Hypoxia is a key regulatory factor in tumor growth, causing hypoxia-inducible factors (HIF) to activate the transcription of genes with crucial roles in tumor biology, including those influencing growth factors, altered glucose metabolism, genomic instability and amplification, invasion, angiogenesis, and metastasis (reviewed in Refs. 4–7). As a result, hypoxia and subsequent genetic interactions may lead to overexpression of HIF and may potentially increase patient mortality.

Rodent Models of Prostate Cancer

There is great need for a model of refractory cancer that could provide leads to mechanisms aimed at prevention and therapy. Current murine models of prostate cancer include: (i) genetically engineered mice, and (ii) five strains of rats: Wistar Unilever; Fischer F344; Noble; ACI; and Lobund-Wistar (LW). A consensus report on 22 genetically engineered mice models expressed cautious optimism that useful systems would be developed, but they need further development (8). However, the transgene adenocarcinoma mouse prostate (TRAMP) transgenic mouse is an excellent model that spontaneously develops metasta-

Table 1. Development of Prostate Cancer in LW Rats[a]

Tumor manifestation	Incidence	Average latency period (months)
Spontaneous	155/516 (30%)	Age 20.8
Induced		
MNU (30 mg/kg body wt)	122/413 (29.5%)	12[b]
MNU + TP × 3	264/303 (87%)	8[b]
Transplanted (PAIII)	1096/1100 (99%)	30 days[b]

[a] Autochthonous tumors developed in the seminal vesicles, anterior prostate, and dorsolateral lobes; metastatic to lungs and/or into the peritoneal cavity. Incidence varied with number of TP implants. All rats were fed diet L485, containing soy meal.
[b] Time after onset of experiment.

sizing prostate cancer that is prevented by genistein (9). Rat models were assessed at the First International Workshop on Animal Models of Prostate Cancer (10): the Wistar Unilever, F344, and Noble rat strains are of limited use because they do not develop HRPC spontaneously; HRPCs are induced in them by extensive and intensive manipulation of chemical carcinogens and prolonged exposures to high doses of testosterone (T) and/or of estrogens. Most of the tumors were of microscopic size, rarely metastatic, and did not progress to the refractory disease described in Table 4. The ACI (AXC) rat strain develops cancer spontaneously in the ventral lobe, a site that is absent in man.

The LW Rat Model of Prostate Cancer

The LW rat was derived from a long-term breeding colony of germfree inbred Wistar strain rats fed a natural ingredient diet, L485 (Teklad, Madison, WI; Ref. 11) *ad libitum*. It was noted, initially in the 37th generation, that occasional aged rats had developed large metastasizing adenocarcinomas in the prostatic complex (12). Large primary HRPCs had developed expanding foci of necrotic cells, especially in the center of the tumors. Metastatic tumors were observed in the lungs and/or in the peritoneal cavity, where the tumor cells attached to and invaded visceral organs. The rats were conventionalized (removed from the germfree isolator) at the 57th generation and maintained pathogen-free thereafter, in isolated environmentally controlled rooms. The incidence of spontaneous metastatic HRPC increased in aged conventionalized rats from approximately 10% to approximately 30%. Transplants of tumor cells and of skin from LW rats were rejected by rats of five inbred rat strains (Wistar, Fischer, Sprague-Dawley, Copenhagen, and Noble strains; unpublished data), suggesting that underlying genetic change(s) had occurred in the LW rat. We do not know why the LW rat appeared in the

Table 3. Progression of Spontaneous Prostate Cancer in LW Rats[a]

Stages of prostate cancer development (age 12 months)	
I Testosterone Dependent	II Testosterone independent
Promoted by:	Refractory to T deprivation by:
DHT benzoate	Castration
TP	NE-DHT
Phenobarbital	Estradiol
	SPII
Prevented by and reversed by:	PAIII transplantable to:
Castration	Female LW rats
Estradiol	Male LW rats
NE DHT	Castrated LW rats
SPII	

[a] NE-DHT, nonesterified DHT; SPII, soy protein isolate/isoflavones.

Table 2. Homology of Spontaneous Prostate Cancer in LW Rats and Patients

Manifestation	LW rats	Patients
Familial	+	+
Age related	+	+
Spontaneous	+	+
Androgen promoted	+	+
Premalignant (PIN)	+	+
Multifocal	+	+
Multistage	+	+
Androgen dependent (early stage)	+	+
Androgen independent (late stage; refractory)	+	+
Adenocarcinoma	+	+
Angiogenesis	+	+
Metastatic	+	+
EZH2 gene expression	+	+
Chemotherapy	−	−

Table 4. Homology of Spontaneous Hormone-Refractory Prostate Cancer in LW Rats and Patients

Manifestation	LW rats	Patients
Spontaneous	+	+
Response to T deprivation	−	−
Response to chemotherapy	−	−
Response to estradiol	−	−
Rapid tumor growth	+	+
Stromal hyperplasia	+	+
Intratumor necrosis	+	+
Intratumor hypoxia	+	+
Intratumor HIF	+	+
Plasminogen activators	+	+
Procoagulant	+	+
Vascular endothelial growth factor	+	+
Metastasis	+	+
EZH2 gene	+	+
Met expression	+	+

colony of germfree Wistar rats, although genetic drift is a likely explanation.

The LW Rat Model of Spontaneous PC—Premalignant Stage I. Our historical record shows that PCs developed spontaneously, with metastatic spread from large advanced-stage tumors in 155/516 (30%) LW rats by average age 22 months (Table 1). Spontaneous PCs in LW rats share developmental characteristics with PCs in aging patients (Table 2). The LW rat is inherently predisposed to develop spontaneous metastasizing PC by endogenous mechanisms through two stages (Table 3). The initial site of tumorigenesis was reported in the seminal vesicle (13) and subsequently reported in the anterior lobe of the prostate (14–16). Some histologic sections demonstrated a primitive branching duct system in the dorsolateral lobes, surrounded by aggregates of large tumor cells with mitotic figures. Increased numbers of cells, many in glandular formation, join with other aggregates to produce the large tumor that we associate with refractory cancer. This suggests that T-dependent cells are activated in the seminal vesicles, in the anterior prostate, and, likely, in the dorsolateral lobes. In the premalignant clinically not apparent stage, luminal cells in the seminal vesicle developed microscopic T-dependent foci of hyperplastic, dysplastic, and neoplastic cells—the likely precursors of prostate cancer (17). The activated luminal cells penetrated the basement membrane into a preformed matrix (Fig. 1), where they multiplied as sheets of large cells with prominent nuclei and assembled to form glandular structures. The driving oncogenic force is associated with high endogenous production of mitogenic T (18, 19), reaching sustained or transient levels in excess of 3000 pg/ml serum. Rats of the five inbred strains noted above that rarely develop PC spontaneously manifested endogenous T levels no higher than 1500 pg/ml of serum (data not published).

The clinical manifestations of PC can be enhanced or suppressed by exogenous agents. For example, tumor development was promoted by high dietary fat (19), by subcutaneous slow-release implants of exogenous T-propionate (20, 21), by exogenous dihydrotestosterone (DHT) propionate (22); and suppressed by caloric restriction (23),

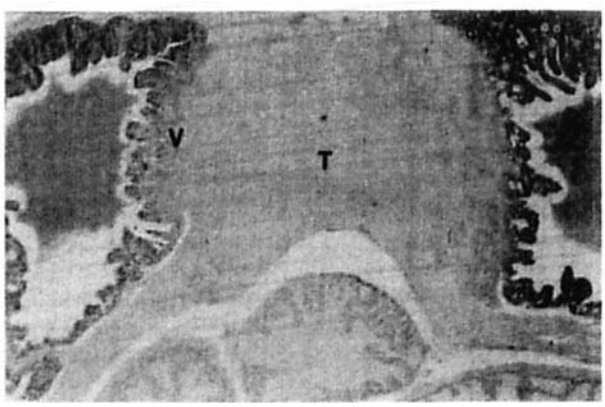

Figure 1. Spontaneous premalignant Stage I prostate tumor (T), developed from testosterone-activated luminal cells in seminal vesicles (V). Hematoxylin and eosin (H&E) stain; magnification: ×40.

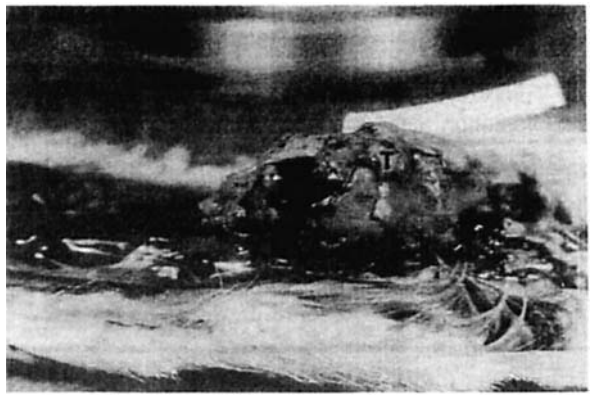

Figure 2. Advanced Stage II HRPC (T) in a 22-month-old LW rat.

by T-deprivation resulting from early castration, by subcutaneous implants of exogenous estradiol, by implants of nonesterified DHT (24), and by dietary soy protein isolate with isoflavones (SPII; Refs. 18, 19, 25). The oncogenic driving force of T on PC development (and spontaneous development of liver tumors in rats older than 30 months) was moderated by dietary restriction (26). Up to age 12 months, progression of most tumors to the refractory Stage II was reversed by changing the diet from L485 to the SPII diet (19). Diet L485 contains soy meal, which did not reduce levels of T, although the amount of isoflavones in soy meal exceeded those in the SPII diet.

Spontaneous Refractory HRPC—Stage II. Without intervention by T-deprivation before age 12 months, premalignant Stage I tumors progressed spontaneously to refractory Stage II tumors. At age 18–20 months, small tumors (approximately 0.5-cm diameter) were palpable in the caudal area of the pelvic region of the abdomen as hard structures with rough surfaces. The tumors grew to approximately 3-cm diameters within the following 2 months (Fig. 2). The large tumors were solid and scirrhotic when incised, rapidly growing, irreversible, and metastatic, with numerous glandular structures (Fig. 3). As the tumors increased in size, they developed three morphological zones after exposure to the pimonidazole reagent: (i) expanding

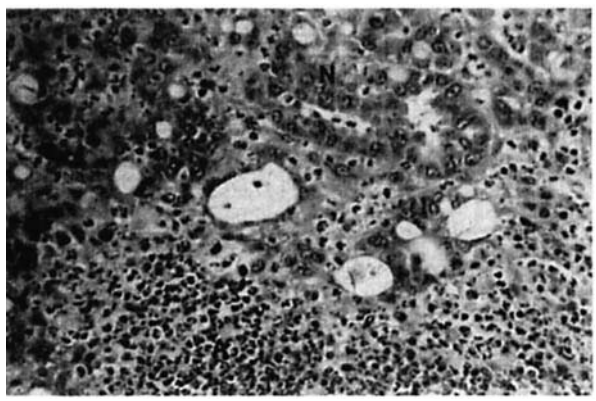

Figure 3. Photomicrograph of advanced Stage II spontaneous refractory prostate cancer. Note the necrotic, anoxic area (A) and normoxic tumor (N). Stained with hematoxylin and eosin (H&E); magnification: ×250.

foci of cellular necrosis in the center of the tumor; (ii) tumor cells adjacent to the necrotic foci demonstrated positive staining for hypoxia; and (iii) normoxic cells in the periphery of the tumors that were replicating and appeared morphologically normal (Fig. 4). During progression, tumor stromas changed from sparse to prominent dense connective tissue; monolayered luminal cells in glands changed to multilayered large tumor cells with prominent nuclei and occasional mitotic figures; and glandular structures appeared in various rigid shapes, with reduced secretions. Tumor cells spread from the primary tumor through lymphatic channels, with occasional swollen lumbar, hepatic, and axillary lymph nodes, to the lungs, where they developed as discrete, expanding, solid subpleural invasive tumors. Tumor cells also penetrated the prostate capsule into the peritoneal cavity, where they developed as pearl-like tumors that attached to peritoneal membranes and invaded visceral organs. The Stage II refractory PCs did not respond to T deprivation. Spontaneous metastasis of tumor cells to skeletal bone was not observed. Obstruction of urine flow by large dorsolateral tumors resulted in urine retention and hydronephrosis.

Table 4 lists characteristics associated with refractory cancer in LW rats and in human patients (4–7), including stromal hyperplasia; intratumor hypoxia, as determined by staining with the pimonidazole reagent; expression of HIF-1a; presence of the tumor-promoting gene EZH2, as determined by gene microarray (presence in LW rat prostate tumors communicated by A. Martin); expression of urokinase plasminogen activator (27); expression of tissue procoagulant factor (28); expression of vascular endothelial growth factor, as determined by immunohistochemistry; and the presence of hepatocyte growth factor/scatter factor tyrosine kinase Met receptor (presence in the transplantable PAIII rat tumor communicated by G. van de Woude; Ref. 29).

Chemically Induced Refractory HRPC in LW Rats. Metastasizing autochthonous HRPCs were induced in LW rats after a single intravenous inoculation of methylnitrosourea (MNU), with or without subsequent subcutaneous implants of T-propionate (TP) in slow-release silastic capsules, each at intervals of 2 months (Table 1). Methylnitrosourea-induced tumors developed in approximately 30% of rats at approximately 12 months after MNU exposure. Among rats treated with MNU plus 2–3 implants of TP, approximately 80% (variable with the number of implants) developed PCs by approximately 8 months after MNU inoculation. Development Stages I and II occurred with shorter latency periods. Reversal of tumorigenesis could be initiated by changing diets from L485 to SPII at 6 months after MNU inoculation; and at 4 months after MNU plus TP inoculations (30). The induced tumors and lung metastases were morphologically similar to the spontaneous refractory HRPCs; and the tumors demonstrated the three morphological zones (including the hypoxia zone stained by the pimonidazole reagent) and most of the characteristics of the refractory disease noted in Table 4.

Occasional rats, representing many strains, including the LW rat, develop local carcinomas in the external ear canal (Zymbal's tumor) in response to MNU inoculation (31).

The PAIII Transplantable Prostate Cancer Cell Line. The PAIII cell line was derived from a germfree LW rat with spontaneous advanced Stage II HRPC. The cells were propagated *in vitro*, cloned, and inoculated subcutaneously into LW rats at 2 months of age. Local and metastatic lung tumors developed (32). The PAIII cells have been transplanted for many passages in LW rats with no change in pattern of disease (33), and the cell line is stored frozen at liquid nitrogen temperature. When PAIII cells were transplanted subcutaneously in the flank, approximately 99% of the rats developed large metastasizing subcutaneous adenocarcinomas within 40 days. The tumors demonstrated the three histological zones related to the intratumor oxygen gradient when stained with the pimonidazole reagent. The PAIII cells spread spontaneously from the subcutaneous primary tumor through ipsilateral lymphatic channels, resulting in numerous coalescing metastatic tumors in the lungs. The refractory PAIII cells produced subcutaneous and metastatic lung tumors in intact male and female LW rats and in castrated LW rats, but not in other rat strains (Table 3). Tumors arising from PAIII cells did not respond to the T-deprivation procedures noted above. We studied the *in vivo* interaction of tumor cells on skeletal bone. The PAIII cells were deposited on the surface of skeletal bone, with disruption of the periosteum. There were numerous osteoclasts at the interface of the PAIII tumor and bone. The PAIII cells invaded the bone, causing osteolytic and adjacent osteoblastic changes, which were prevented by subcutaneous inoculations of dichloromethylene diphosphonate. However, this drug had no effect on established osteoblastic lesions. The PAIII cells did not pass through the intact periosteum (34–36).

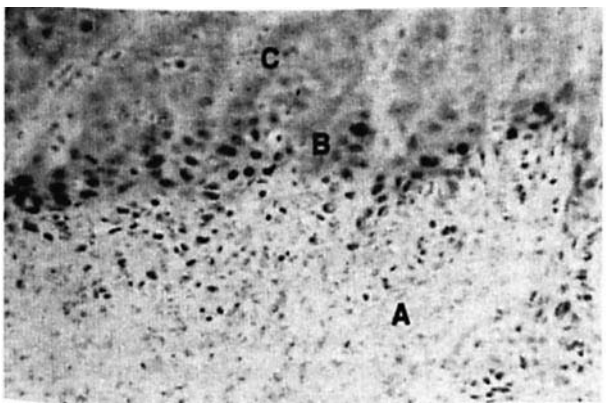

Figure 4. Photomicrograph of spontaneous refractory prostate cancer. Note three zones: anoxic, necrotic zone (A); hypoxic zone (B); and normoxic zone (C). Stained with pimonidazole hypoxyprobe; magnification: ×250.

Discussion

The goal of cancer prevention must focus on the refractory disease, the terminal stage that develops in many organ-related solid cancers. Hormone-refractory prostate cancer is a good example of that disease. Most of the autochthonous prostate tumors induced by intensive doses of chemical and hormone agents in Fischer (37) and in Wistar Unilever (38) rats produced premalignant tumors that did not progress to the refractory stage of tumorigenesis (Table 4); therefore, these models would be inappropriate for studies on prevention and therapy of refractory cancer.

The versatility of the four experimental protocols provided in the LW model system (Table 1) has attracted many investigators who represent a wide range of disciplines in basic and applied subjects. Some of their published reports include: siRNA (39); tumor biology (15, 40–43); antiangiogenesis (22); electron microscopy (44); genomic linkages (45); metastasis (46–49); therapy (50–55); immunization (56, 57); tumor prevention (58–60); caloric restriction (61); bone cancer (62–65); androgen receptor (66); procoagulant activity (28); and the EGF growth promoter (67).

Development of prostate cancers was associated with stromal changes (called stromal tumors) in cancers in the breast and prostate (68). Subsequently, this lesion was associated with stromal-epithelial interchanges in a variety of cancers that were associated with the hepatocyte growth factor/scatter factor tyrosine kinase Met receptor, causing rapid tumor growth and metastasis (69). The Met receptor is expressed by PAIII cells and manifests the related stromal hyperplasia noted above. Is it possible that inhibition of tyrosine kinase by genistein will inhibit development of the Met receptor?

This is the first documented model of HRPC that *will* enable studies on prevention and therapy. The relationship of hormone-refractory cancer may be related mechanistically to refractory disease associated with chemical- and radiation-treated cancers. Supporting this notion is our finding that the premalignant stage of induced PC was significantly prevented in LW rats by dietary 4-hydroxyphenylretinamide (4-HPR; Ref. 70), and feeding the same 4-HPR to male LW rats did not significantly inhibit the growth and spread of the hormone-refractory PAIII cells (71).

In summary, a growing body of evidence shows that the LW rat is a unique animal model for studies aimed at prevention and therapy of HRPC.

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1. Furth J, Kim U, Clifton KH. On evolution of the neoplastic state; progression from dependence to autonomy. Bethesda, MD: National Institutes of Health, National Cancer Institute Monograph No. 2 149–177, 1960.
2. Thomlinson RH, Gray LH. The biological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer 9:539–549, 1955.
3. Raleigh JA, Calkins-Adams DP, Rinkert H, Bellinger CA, Weissler MC, Fowler WC. Hypoxia and vascular endothelial growth factor expression in human squamous cell carcinomas using pimonidazole as a hypoxia marker. Cancer Res 58:3765–3768, 1998.
4. Semenza GL, Wang WG. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 12:5447–5454, 1992.
5. Harris AL. Hypoxia—a key regulatory factor in tumor growth. Nature Rev Cancer 2:38–46, 2001.
6. Rockwell S, Yuan J, Pereta S, Glazer PM. Genomic instability in cancer. Novartis Found Symp 133–151, 2001.
7. Edwards J, Krishna S, Witton CJ, Bartlett JMS. Gene amplifications associated with the development of hormone resistant prostate cancer. Clin Cancer Res 9:5271–5281, 2003.
8. Shoppell SB, Thomas GV, Roberts RL, Herbert R, Itemann MM, Rubin MA, Humphrey PA, Sundberg JP, Rozengurt N, Barrios R, Ward JR, Cardiff RD. Prostate pathology of genetically engineered mice: definition and classification. The consensus report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. Cancer Res 64:2270–2305, 2004.
9. Mentor-Marcel R, Lamartiere CA, Eltoun IE, Greenberg NM, Elgavish A. Genistein in the diet reduces the incidence of poorly differentiated prostatic adenocarcinoma in transgenic mice (TRAMP). Cancer Res 61:6777–6782, 2001.
10. Bostwick DG (Rappõteur). Workshop I. Rodent models of prostate cancer. Prostate 36:49–55, 1998.
11. Kellog TF, Wostmann BS. Stock diet for colony production of germfree rats and mice. Lab Anim Care 19:812–814, 1969.
12. Pollard M. Spontaneous prostate adenocarcinomas in aged germfree Wistar rats. J Natl Cancer Inst 51:1235–1241, 1973.
13. Hoover DM, Best KL, McKenny BK, Tamura RM, Neubauer BL. Experimental induction of neoplasia in the accessory sex organs of male Lobund Wistar rats. Cancer Res 50:142–146, 1990.
14. Pollard M, Luckert PH. Early manifestations of induced prostate tumors in Lobund-Wistar rats. Cancer Lett 67:163–166, 1992.
15. Cohen MB, Heidger PM, Lubaroff DM. Gross and microscopic pathology of induced prostatic complex tumors arising in Lobund Wistar rats. Cancer Res 54:626–628, 1994.
16. Tamano S, Rehm S, Waalkes MP, Ward JM. High incidence of seminal vesicle adenocarcinoma and lower incidence of prostate carcinomas in the Lobund-Wistar prostate cancer rat model using n-nitrosomethylurea and testosterone. Vet Pathol 33:557–567, 1996.
17. Bostwick DG. High grade prostatic intraepithelial neoplasia. Cancer 75(Suppl):1823–1836, 1995.
18. Pollard M, Wolter W. Prevention of spontaneous prostate-related cancer in Lobund-Wistar rats by a soy protein isolate/isoflavone diet. Prostate 45:101–105, 2000.
19. Pollard M, Wolter W, Sun L. Diet and the duration of testosterone-dependent prostate cancer in Lobund-Wistar rats. Cancer Lett 173:127–131, 2001.
20. Pollard M, Luckert PH. Promotional effects of testosterone and high fat diet on the development of autochthonous prostate cancer in rats. Cancer Lett 32:223–227, 1986.
21. Pollard M, Luckert PH, Snyder DL. The promotional effect of testosterone on induction of prostate cancer in MNU-sensitized L-W rats. Cancer Lett 45:209–212, 1989.
22. Joseph BJK, Vukanovic J, Isaacs JT. Antiangiogenic treatment with linomide as chemoprevention for prostate, seminal vesicle and breast carcinogenesis in rodents. Cancer Res 56:3404–3408, 1996.
23. Snyder DL, Pollard M, Wostmann BS, Luckert PH. Lifespan,

morphology, and pathology of diet-restricted germ-free and conventional Lobund-Wistar rats. J Gerontol Biol Sci 45:B52–B58, 1990.
24. Pollard M, Luckert PH, Snyder D. Prevention and treatment of experimental prostate cancer in Lobund-Wistar rats. I. Effects of estradiol, dihydrotestosterone and castration. Prostate 15:95–103, 1989.
25. Pollard M, Wolter W, Sun L. Prevention of induced prostate-related cancer by soy protein isolate-isoflavone-supplemented diet in Lobund-Wistar rats. In Vivo 14:389–392, 2000.
26. Pollard M, Luckert PH, Snyder D. Prevention of prostate cancer and liver tumors in LW rats by moderate dietary restriction. Cancer 64:686–690, 1989.
27. Strickland DK, de Serrano VS, Chan S-Y, Pollard M, Castellino FJ. Purification and properties of a plasminogen activator from cultured rat prostate adenocarcinoma cells. Biochemistry 22:4444–4449, 1983.
28. Adamson AS, Luckert PH, Pollard M, Snell ME, Amirkhostari M, Francis JL. Procoagulant activity may be a marker of the malignant phenotype in experimental prostate cancer. Br J Cancer 69:286–290, 1994.
29. Birchmeier C, Birchmeier W, Ghorardi E, Van de Woude G. MET, metastasis, motility, and more. Nature Rev Mol Cell Biol 4:915–925, 2003.
30. Pollard M. The Lobund-Wistar rat model of prostate cancer. J Cell Biochem 16H(Suppl):84–88, 1992.
31. Zymbal WE. Histologische und experimentelle untersuchen am epithelegewebe der talgdrasen (gehorgangdruse der ratte) [Histological and experimental studies of epithelial tumors of the ear canal in the rat]. Z Zellforsch 18:596–625, 1933.
32. Chang CF, Pollard M. In vitro propagation of prostate adenocarcinoma cells from rats. Invest Urol 14:331–334, 1977.
33. Pollard M, Luckert PH. Transplantable metastasizing prostate adenocarcinoma in rats. J Natl Cancer Inst 54:643–649, 1975.
34. Pollard M, Luckert PH. Effects of dichloromethylene diphosphonate on the osteolytic and osteoblastic effect of rat prostate adenocarcinoma cells. J Natl Cancer Inst 75:949–954, 1985.
35. Pollard M, Luckert PH. The beneficial effects of diphosphonate and piroxicam on the osteolytic and metastatic spread of rat prostate carcinoma cells. Prostate 86:882–886, 1986.
36. Pollard M, Luckert PH, Scheu J. Effects of diphosphonate and x-rays on bone lesions induced in rats by prostate cancer cells. Cancer 61:2027–2032, 1988.
37. Shirai T, Tamano S, Kato T, Iwasaki S, Takahashi S, Ito N. Induction of invasive carcinomas in the accessory sex organs other than the ventral prostate of rats given 3,2′-dimethyl-4-aminobiphenyl and testosterone propionate. Cancer Res 51:1264–1269, 1991.
38. Boiteau TW-M, Liao Z, Kim S, Lameshow S, Erdman JW, Clinton SK. Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. J Natl Cancer Inst 95:1578–1586, 2003.
39. Burnfeind P. Antisesense RNA to the type 1 insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells in vivo. Proc Natl Acad Sci U S A 93:7263–7268, 1996.
40. Djakiew D, Tarkington MA, Lynch JH. Paracrine stimulation of polarized secretion from monolayers of a neoplastic prostatic epithelial cell line by prostatic stromal cell proteins. Cancer Res 50:1966–1974, 1990.
41. Danielpour D, Kadomatsu K, Anzano MA, Smith JM, Sporn MG. Development and characterization of nontumorigenic and tumorigenic epithelial cell lines from a rat dorso-lateral prostate. Cancer Res 54:3413–3421, 1994.
42. Slayter MV, Anzano MA, Kadomatsu K, Smith JM, Sporn MB. Histogenesis of induced prostate and seminal vesicle carcinoma in Lobund-Wistar rats: a system for histological scoring and grading. Cancer Res 54:1440–1445, 1994.
43. Hodde JP, Suckow MA, Wolter WR, Hiles MC. Small intestinal submucosa does not promote PAIII tumor growth in Lobund-Wistar rats. J Surg Res 120:189–194, 2004.
44. Heidger PM. Fine structural studies of induced tumors arising within the prostatic complex of Lobund-Wistar rats. Prostate 32:173–178, 1997.
45. Schlicht M, Matysiak B, Brodzeller T, Wen X, Liu H, Zhou G, Dhir R, Hessner M, Tonellato P, Suckow M, Pollard M, Datta M. Cross-species global and subset gene expression profiling identifies genes involved in prostate cancer response to selenium. BMC Genomics 5:58, 2004.
46. Neubauer BL, Bemis KG, Best KL, Goode RL, Hoover DM, Smith GF, Tanzer LR, Merriman RL. Inhibitory effect of warfarin on the metastasis of the PAIII prostatic adenocarcinoma in the rat. J Urol 135:163–166, 1986.
47. Faas FH. Increased phospholipids fatty acid remodeling in human and rat prostatic adenocarcinoma tissues. J Urol 156:245–248, 1996.
48. Neubauer BL, Merriman RL, Best KL, Goode RL, Sundboom J, Tanzer LR, Merriman RL. Anticoagulant and inhibitory effects on the lymphatic metastasis of the PAIII prostatic adenocarcinoma in male rats by recombinant DNA-derived hirudin (THs). Cell Pharmacol 1:113–119, 1994.
49. Neubauer BL, Bemis KG, Best KL, Goode RL, Merriman RL, Smith GF, Tanzer LR, Hoover DM. Metastatic spread of the PAIII prostatic adenocarcinoma after implantation in the tail of the rat. Prostate 8:265–276, 1986.
50. Neubauer BL, Best KL, Counts DF, Goode RL, Hoover DM, Jones CD, Saroody MF, Shaar CJ, Tanzer LR, Merriman RL. Raloxifine (2156758) produces anti-metastatic responses and extends survival in the PAIII rat prostatic adenocarcinoma model. Prostate 27:220–228, 1995.
51. Neubauer BL, McNulty AM, Chedid M, Chen K, Goode RL, Johnson MA, Jones CD, Krishnan V, Lynch R, Osborne HE, Graff JR. The selective estrogen receptor modulator triclofene (L4133314) inhibits metastasis and extends survival in the PAIII rat prostatic carcinoma. Cancer Res 63:6056–6062, 2003.
52. Torosian MH, Donoway RB. Growth hormone inhibits tumor metastasis. Cancer 67:2280–2283, 1991.
53. Neubauer BL, Best RL, Clemens JA, Gates C, Goode RL, Jones CD, Shaar CJ, Hoover DM. Endocrine and anti-prostatic effects of raloxifene (L4156758) in the male rat. Prostate 23:245–262, 1993.
54. Neubauer BL, Best RL, Goode RL, Heiman ML, Robertson DW, Saroody MF, Sloan MF, Tanzer LR, Merriman RL. Comparative anti-tumor effects of hormone ablation, estrogen agonist cytotoxic derivative and anti-estrogen in the PAIII rat prostatic adenocarcinoma. Cancer Res 52:4663–4671, 1992.
55. Arcadi JA. Studies of rhodamine-123 effect on rat prostate and human prostate cancer cells in vitro. J Surg Oncol 59:86–92, 1995.
56. Hrouda D, Souberbielle BE, Kayaga J, Corbishley CM, Kirby RS, Dalgleish AG. Mycobacterium vaccae (SRL 172): a protective immunological adjuvant evaluated in rat prostate cancer. Br J Urol 82:870–876. 1998.
57. Hrouda D. Allogeneic whole tumor cell vaccination in the rat model of prostate cancer. Br J Urol 86:742–748, 2000.
58. Lucia MS, Anzano MA, Slayter MV, Anver MR, Green DM, Shrader MW, Logsdam DL, Driver CL, Brown CC, Peer CW, Roberts AB, Sporn MB. Chemopreventive activity of tamoxifen, n-(4-hydroxyphenyl) retinamide, and the vitamin D analogue R024-5531 for androgen-promoted carcinomas of the rat seminal vesicle and prostate. Cancer Res 55:5621–5627, 1995.
59. Wang J, Eltoum I-E, Lamarteniere CA. Dietary genistein suppresses chemically induced prostate cancer in Lobund-Wistar rats. Cancer Lett 186:11–18, 2002.
60. Lamarteniere CA. Genistein chemoprevention timing and mechanisms of action in murine mammary and prostate. J Nutr 132:5525–5585, 2002.
61. Chen LH. Effects of age and dietary restrictions on liver glutathione transferase activities in Lobund-Wistar rats. Arch Gerontol Geriatr 18:191–205, 1994.
62. Koutsilieris M. PA-III rat prostate adenocarcinoma cells (Review). In Vivo 6:199–204, 1992.

63. Koutsilieris M, Polychronakos MC. Proteinolytic activity against IGF-binding proteins involved in the paracrine interactions between prostate adenocarcinoma cells and osteoblasts. Anticancer Res 12:905–910, 1992.
64. Koutsilieris M. Urokinase-type plasminogen activator: a paracrine factor regulating the bioavailability of IGFs in PAIII cell-induced osteoblastic metastases. Anticancer Res 13:481–486, 1993.
65. Reyes-Moreno C. Glucocorticoid function possibly modulates cell-cell interactions in osteoblastic metastases on rat skeleton. Clin Exp Metast 15:205–217, 1997.
66. Bentel JM, Pickering MA, Pollard M, Clements JA, Tilley WD. Androgen receptor expression in primary prostate cancer of Lobund-Wistar rats and in tumor-derived cell lines. Cell Dev Biol Animal 35:655–662, 1999.
67. Dalu A, Haskell JF, Coward L, Lamartiniere CA. Genistein, a compound of soy, inhibits the expression of the EGF and ErB2/neu receptors in the rat dorsolateral prostate. Prostate 37:36–43, 1988.
68. Grossfeld GD, Howard SW, Tisty TD, Cunha GR. The role of stroma in prostate carcinogenesis. Endocr Rel Cancer 5:253–270, 1998.
69. Knudsen BS, Edlund M. Prostate cancer and the Met hepatocyte growth factor receptor. Adv Cancer Res 91:31–67, 2004.
70. Pollard M, Luckert PH, Sporn MB. Prevention of primary prostate cancer in Lobund-Wistar rats by n-(4-hydroxyphenyl) retinamide. Cancer Res. 51:3610–3611, 1991.
71. Pollard M, Luckert PH. The inhibitory effect of 4-hydroxyphenyl retinamide (4HPR) on metastasis of prostate adenocarcinoma-III cells in Lobund-Wistar rats. Cancer Lett. 59:159–163, 1991.