Prostatic Localization of Spontaneous Early Invasive Carcinoma in Lobund-Wistar Rats

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Abstract

Animal models of human prostate cancer are very limited in number but are of obvious importance to develop. Dr. Morris Pollard (M. Pollard, J. Natl. Cancer Inst., 51: 1235—1241, 1973) has reported that Lobund-Wistar rats develop spontaneous metastatic prostatic cancer when they become old (—25% incidence after 25 months). A chemically induced form of the disease has also been described in Lobund-Wistar rats. However, recent reports suggest that most of the chemically induced adenocarcinomas are not prostatic in origin, with most arising in the seminal vesicle, and thereby raise questions about the origin of the spontaneous cancers. We herein report cancer spontaneously arising in the lateral lobes of the prostates in Lobund-Wistar rats. One of 8 rats killed at 16 months of age showed prostatic carcinoma in situ. Two of 39 rats killed at 20 months displayed early invasive adenocarcinomas with no signs of metastases. Because sectioning of the prostates in this study was limited to face sections from a single block for each rat, it is highly probable that the true incidence of dysplasias and carcinomas is underestimated by these data. Dysplastic or neoplastic changes were not seen in either the seminal vesicles or other portions of the prostatic complex. The nuclei of adenocarcinoma cells showed less labeling with antibody to the androgen hormone receptor than did normal cells. These data strongly support the validity of the Pollard model of spontaneous prostate cancer in Lobund-Wistar rats.

Introduction

A 1991 summary report (1) of the “National Cancer Institute Roundtable on Prostate Cancer: Future Research Directions” emphasized the need to develop appropriate animal models of human prostate cancer. The Lobund-Wistar (L-W) rat, studied by Pollard and his colleagues (2, 3), is one of three rat models of spontaneous prostate cancer. The L-W rat is unique among the rat models and potentially advantageous because the prostate cancer reportedly occurs spontaneously in —25% of old animals and is metastatic. L-W rats also develop a chemically induced form of the disease. However, a recent report (6) stated that most (73%) of the chemically induced adenocarcinomas originate in the seminal vesicle (and not in the prostate). Other reports (7, 8) also show that the chemically induced tumors can arise in nonprostatic urogenital tissues. Together, these findings, coupled with the fact that Pollard’s experiments (2) on the spontaneous cancer did not utilize timed autopsies to study the early stages of the disease, raise questions about the site of origin of the spontaneous cancer. In the present study, L-W rats were killed at 3, 16 and 20 months of age and examined for the histopathological status of the prostatic complex. In this report we describe the occurrence of early invasive carcinomas confined to the lateral lobe of prostates from L-W rats.

Materials and Methods

Experimental Design. Fifteen-month-old male L-W rats were purchased from Hilltop Lab Animals (Scottsdale, PA). Other young (1-month-old) male L-W mice were purchased from the same source and used later as young (3-month-old) controls. While at Hilltop Lab Animals the rats were housed two per cage and given free access to Agway RMH 3500 rat chow. Upon arrival in Madison, the rats were single caged with filter tops at the American Association for Accreditation of Laboratory Animal Care-certified Shared Aging Rodent Facility at the VA Medical Center. At their arrival and monthly thereafter, sentinel rats were certificated free of pathogenic viruses and Mycoplasma. The rats continued to receive the Agway RMH 3500 diet ad libitum until reaching 19 months of age. With the exception of the dietary regimens after 19 months of age, the rats were maintained under conventional conditions: 24 ±2°C, 50 ±10% relative humidity, 12—16 air cycles of air exchange/h, 12-h light/12-h dark light cycle, and free access to acidified water. After the 19th month of age, the rats were divided into four groups and fed one of four diets as part of a larger study on the effects of changes in calorie and/or fat intake started in late-middle age on the development of prostate cancer. These diets are not detailed herein because only one month of feeding occurred by 20 months of age and intergroup differences in prostate histology or cancer were not apparent at this age. Rats were killed at three ages, 3 months (“young,” n = 8), 16 months (“middle age,” n = 8), and 20 months (“late-middle age,” n = 39), with an overdose of pentobarbital and the organs were harvested immediately. Organ weights were recorded for the prostatic complex, the seminal vesicles (including the coagulating gland), testes, and several other organs. The weighed samples were flash frozen in liquid nitrogen for subsequent biochemical analyses.

Histopathological Evaluation. A segment of the prostatic complex from each rat, comprising a representative sample of the dorsolateral lobes and the urethra, and cross-sectional cuts from the middle portions of both seminal vesicles with the adhering coagulating gland (anterior lobe of the prostate) were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2—3 h, dehydrated in a graded series of ethanol and embedded in paraffin. Remaining portions of the prostatic complex were frozen in liquid N2 for later molecular analysis. Ten sections from each prostate were cut at 4—5 μm, dewaxed, and stained with hematoxylin and eosin or used for immunohistochemistry (see below).

Immunohistochemistry. Sections cut at 4—5 μm were dewaxed, and pretreated with Tissue Unmasking Fluid (Signet Laboratories, Dedham, MA), heated in a water bath at 85°C for 25 min, and then removed from the bath and cooled for 10 min. Sections were rinsed in PBS at room temperature and incubated with polyclonal rabbit anti-androgen receptor overnight at 4°C in a humidified chamber. The polyclonal antibody used in this study was raised against the purified human androgen receptor peptide (40% of the N-terminal domain and 25% of the DNA-binding domain) from E. coli. Its specificity was confirmed by sucrose gradient centrifugation and immunoprecipitation assays (9, 10). Sections were rinsed in PBS and a biotinylated secondary antibody (goat anti-rabbit IgG) applied for 0.5 h. After rinsing with PBS, the localization of the antibodies was visualized with avidin-biotin

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4 The abbreviations used are: L-W, Lobund-Wistar; PBS, phosphate-buffered saline; AR, androgen receptor.
complex solution (Vector Corp., Burlingame, CA) and developed with metal enhanced 3,3′diaminobenzidine-H2O2 (Pierce Chemical Co., Rockford, IL). Control sections were incubated in the absence of primary antibody and were always negative.

**Statistical Analysis.** Body weights and the weights of the prostate and seminal vesicle at 3 and 20 months are reported as means. Statistical comparisons were performed using Student’s t test. Intergroup differences were considered to be statistically significant when P < 0.05.

**Results**

**Body, Prostate and Seminal Vesicle Weights**

Body weights increased by 2.0 fold, from 278 g at 3 months to 545 g at 20 months. Prostate weights increased from 0.34 to 0.80 g, and seminal vesicle weight increased from 0.45 to 0.83 g over this time period. The increases in body weights and organ weights (prostate and seminal vesicles) with age were all statistically significant (P < 0.0001).

**Prostatic Histopathology**

**Young Rats.** Alveoli of the lateral lobes of the prostates of 3-month-old rats consisted of single cell-thick layers of cuboidal and columnar epithelial cells (Fig. 1A). These cells had the appearance typical of ductal tissue with basal nuclei containing small, infrequent nucleoli. Underlying the cuboidal cells there were inconspicuous basal cells, noted only by the flattened nuclei observed between the epithelial cells and the layer of smooth muscle. In cells of the columnar epithelium, vesicles consistent with secretory activity were observed at the apical poles. Adjacent acini comprised of cuboidal epithelial cells had a less active secretory appearance, although secretory protein often filled the lumens of the acini. The basement membrane on which the epithelial cells lie was immediately surrounded by a thin layer of smooth muscle. These acini lie within a loose stromal matrix of collagen.

**Middle-aged Rats.** By 16 months of age, the lateral lobes in the prostates of all 8 rats exhibited mild hyperplastic elaboration of the columnar epithelium. Marked infoldings of the epithelium in many alveoli protruded into the luminal spaces (Fig. 1B), occasionally dividing the duct into separate areas. This hyperplastic epithelium followed the contours of the alveoli and did not distort the basic structure. Secretory activity at the luminal surfaces of some of the alveoli appeared to be increased. The columnar epithelium remained a single cell-thick layer without significant dysplasia. The nucleoli remained small and were not notably more numerous. There was no apparent increase in mitotic activity. The hyperplastic increase in prostatic epithelium was not a universal feature of all acini as some areas remained quiescent, presenting a cuboidal epithelium.

**Late Middle-aged Rats.** At 20 months of age, the prostatic epithelium continued to exhibit marked hyperplasia and had begun to display increasing signs of dysplasia. Of the 39 rats studied at this age, 100% showed hyperplasia. Occasional piling up of epithelial cells was seen along with a few nests of cells with reduced basophilia (not shown). Hyperplastic epithelium continued to protrude into the lumina of the acini.

Accumulation of neutrophils which filled the luminal space was observed at 20 months in scattered acini from 12 of the 39 rats. We also observed accumulations of chronic inflammatory cells (mostly lymphocytes) in 21 of the 39 rats. Some of these cells occurred as stromal accumulations (Fig. 1C) whereas others occurred mixed with epithelial cells in the prostatic acini.

Examination of the dorsal lobes, which are always observed in the sections along with the lateral lobes, and the ventral lobes, which are occasionally seen in these sections, showed no hyperplasia in these
lobes up to 20 months of age. Furthermore, examination of separate sections from the seminal vesicles and of the coagulating glands (anterior lobes) of these rats revealed no hyperplasia at this age.

**Prostatic Carcinomas**

Sections 2 of the 39 rats sacrificed at 20 months showed early, invasive carcinomas in acini of the lateral lobes. It is highly probable that the true incidence of dysplasias and carcinomas is underestimated by these data because sectioning of the prostates was limited to 10 serial sections from each rat. Fig. 1C shows dysplasia throughout one acinus and the localized anaplasia at one end of the acinus. Surrounding the acinus are glands with the commonly seen hyperplastic epithelium. Chronic inflammatory lymphocytes have infiltrated the stroma and abut the acinus at the site of the invasion of the carcinoma (Figs. 1C and 2A).

The invasive epithelial cells (Figs. 2A and 2C), here characterized by the pale basophilic cytoplasm, were seen outside the acinus and the nearby erosion of the smooth muscle layer by which the invasive cells have left the acinus was apparent. Chronic inflammatory cells were abundant at the site of the invasive carcinoma. Within the acinus, two populations of dysplastic cells were seen. Cells with cytoplasm and nuclei exhibiting reduced basophilia and nuclei with prominent nucleoli were located adjacent to the basement membrane. More normally staining cells were observed in a mass distal to the smooth muscle while the hyperplastic cells (common to the acini of rats at this age) were found in the remainder of the acinus.

A second invasive carcinoma from another rat is shown in Fig. 2B. The carcinoma formed in the basal layers of the hyperplastic epithelium and eroded the basement membrane and the surrounding smooth muscle (Fig. 2B, arrowheads mark the frayed ends of the smooth muscle layer). Cells of the carcinomas have cytoplasmic and occasional nuclei with reduced basophilic staining (Fig. 2D). Invasive cells have mixed with the fibroblastic and stromal cells between the adjacent acini. The overlying layer of prostatic cells in the acinus maintained a somewhat normal staining appearance, although it is clearly hyperplastic. Neutrophils and cellular debris completely filled the lumen of the acinus.

**AR Immunohistochemistry**

Immunostaining for AR showed the expected nuclear localization of this protein in the common hyperplastic epithelium (Fig. 2E). Similar strong nuclear localization of AR was seen in the normally basophilic, but dysplastic cells, at the left of the field. In the area of the carcinoma with reduced basophilia, the nuclear staining was also markedly reduced. Note that the chronic inflammatory cells in Fig. 2, A and C, lacked nuclear AR staining. The second carcinoma’s pattern of AR staining (Fig. 2F) resembled that of the first one. Within the carcinoma, the nuclear staining was markedly reduced. Support cells of the stroma showed AR staining whereas the neutrophils in the lumen lacked AR staining. In both carcinomas, the decreased nuclear staining was associated with an apparent increase in cytoplasmic staining. Whether this alteration represents an intracellular redistribution of AR is unknown.

**Discussion**

Our findings extend the earlier reports of Pollard and his colleagues (2, 3) on spontaneous prostatic cancer in L-W rats. In the first report (2), four adenocarcinomas of the prostate were described in rats maintained under germ-free conditions. One was found in a 22-month-old rat. The other three tumors were found in 32–40-month-old rats (n = 31). The tumors occupied a large part of the pelvic cavity and formed irregular, solid (but soft) masses of white tissue. The anatomical divisions of the prostate were not distinct at the time of autopsy due to the growth of the tumors. Three of the tumors were metastatic to the lungs and two of these had also spread to the liver, spleen, kidney and peritoneum. One tumor did not metastasize.

Leukocytes were present in all of the tumors. In the second report (3), the incidence of metastatic prostate cancer was 26% in a population of conventionally housed L-W rats. The average age when prostate cancer was found was 27 months. The tumors were large (5–40 g). Although it was stated that the tumors developed in the dorsolateral lobes and expanded to the rest of the prostate and to the seminal vesicles, the experimental design of both studies precluded gaining definitive information on the primary site of origin of the tumor and the diagnosis of in situ prostatic adenocarcinomas was “...omitted from the data in Table 1 because such lesions were difficult to define.” Accordingly, to our knowledge, our study is the first to demonstrate and begin to characterize a primary prostatic localization of the early lesion in L-W rats.

There are differences between the L-W rat model of prostate cancer and other rat models of the disease. In 1963, Dunning (11) described a spontaneous prostatic adenocarcinoma which did not metastasize in a 22-month-old retired breeder from the Copenhagen rat strain. The tumor occupied a large portion of the lower abdominal cavity and was thought to have originated in the dorsal prostate. This tumor was transplantable and allowed the development of several tumor cell lines (5); however, subsequent study of spontaneous prostate cancer in Copenhagen rats has apparently ceased. In 1975, Shain et al. (12) reported that spontaneous adenocarcinomas occurred in the ventral prostate of 7 of 41 old (~35-month-old) A × C rats. These were nonmetastatic tumors. The proliferating cells showed marked atypia and were arranged in cribriform patterns as they expanded into the affected glands. A later report (13) on ACI/segHapBR rats (stated to be “...A × C with Irish marker”) confirmed and extended this finding. By 33 months, ~95% of the rats showed intraalveolar atypical hyperplasias and ~35% had locally invasive (but not metastatic) carcinomas. F344 rats have also been found to develop prostatic hyperplasia and neoplasia (14). These neoplasias were rare (4% incidence), nonmetastatic, and involved the ventral prostate.

We have found prominent hyperplasia of the columnar epithelium in the lateral lobes by 20 months of age and early neoplasia in the acini of two rats of this age. It is likely that the incidence of dysplasias and carcinomas is far greater than these data suggest, since sectioning of the prostates in this study was not exhaustive. In contrast to the spontaneous adenocarcinomas found in the ventral lobes of A × C rats (12) and to those found in Fisher 344 rats (14), neither of the carcinomas in the present report has a characteristic cribriform appearance. This is consistent with previous reports of prostatic tumors in the L-W rat (2, 3) in which the cribriform appearance is not described.

A system for histological scoring and grading of dysplasias and neoplasms for chemically induced carcinomas in the L-W rat has recently been proposed (15). Using these criteria, the dysplasias in the present study would appear to be classified as category A, stage 1 and the two carcinomas as category B, stage 3. As more spontaneous carcinomas become available in our study, we will evaluate the utility of this grading system for this model. With regard to classification schemes for human prostate cancer, the hyperplasia we observed is similar in histological appearance to human grade 2 prostatic intraepithelial neoplasia whereas the appearance of the invasive carcinoma is similar to grade 3 prostatic intraepithelial neoplasia (16).

There was acute inflammation in the acini of the lateral lobes, characterized by mild to severe infiltrates of neutrophils, first
Fig. 2. Two invasive carcinomas from L-W rats at 20 months. A, higher magnification of the same carcinoma (CA) as in Fig. 1C. Invasive cells (arrows) have escaped the acinus by penetrating the smooth muscle layer (S). B, invasive carcinoma (CA) from a second 20-month-old rat. The carcinoma is under a layer of hyperplastic epithelial cells. Ends of the smooth muscle (arrowheads) show the invasion of the stroma. Neutrophils (N) fill the lumen of the gland. C, enlargement of area indicated by the box in A. Cancerous cells (arrow) have large, pale basophilic nuclei with prominent nucleoli. Smooth muscle of acini (arrowhead). D, enlargement of the area indicated by the box in B, showing the anaplastic cells of the invasive carcinoma where the smooth muscle (arrowhead) has been eroded. E, immunostaining of carcinoma in A for androgen receptor. Strong nuclear staining in the non-neoplastic epithelium (right) and in many of the dysplastic cells (upper left). There is a striking loss of nuclear AR staining in the carcinoma (CA) at the point of invasion of the stroma. F, immunostaining for AR of carcinoma in B. Nuclei of cells in adjacent acini and in cells of the stroma are stained positively for AR. Magnifications: A, B, E, and F, bar, 20 µm; C and D, bar, 14 µm.
observed in the 16-month-old rats and increasingly prominent at 20 months. In one case, the acinus with invasive carcinoma was filled with acute inflammatory cells, and in the other case, chronic inflammatory cells (predominantly a lymphocytic infiltrate) were found in the stroma adjacent to the carcinoma. Pollard et al. (3) had reported that prostatitis appeared prior to age 20 months in 20% of their L-W rats. In aging F344 rats, prostatitis was seen in addition to neoplasia but the authors suggested there was a lack of association between the inflammation and neoplasia (14). The extent to which the presently reported inflammation is causally related to prostate cancer in L-W rats is presently under investigation.

We have shown that the AR is strongly positive by immunohistochemistry in nuclei of the prostatic epithelium in all lobes of L-W rats and that the nuclear expression is reduced in the two invasive carcinomas seen at 20 months of age. This nuclear localization is consistent with the report of Takeda et al. (10) who first demonstrated the nuclear immunohistochemical localization of AR in the prostatic epithelium of rats (Sprague-Dawley strain), mice, and humans. Staining intensity of AR is known to vary among the lobes of the rat prostate, with a pattern of ventral > lateral > dorsal lobe staining in Sprague-Dawley rats (17). We are unaware of previous studies of the AR staining status of spontaneous prostate cancers harvested from aged rats; however, the AR staining properties of human prostate cancer cells have been extensively studied. Sections from human prostate adenocarcinomas show a decreased staining intensity and increased heterogeneity of AR expression as compared to normal prostate epithelial cells (18–21). The extent to which the L-W model resembles human prostate cancer in AR staining properties cannot be established from the present data on two very early cancers; however, ongoing studies of tumors from our aging cohort of L-W rats should prove informative in this regard.

The present findings provide new information on the early events associated with the spontaneous development of prostate cancer in L-W rats. The neoplasm is preceded by hyperplasia of the glandular epithelium starting in middle age. Both the hyperplasia and neoplasia occur in the lateral part of the gland. Also, the two cases of early prostatic carcinoma reported herein were associated with the presence of acute or chronic inflammatory infiltrates and with a decreased expression of the AR. These data support the validity of the L-W rat as a model for the study of spontaneous prostate cancer.

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