A Probasin-Large T Antigen Transgenic Mouse Line Develops Prostate Adenocarcinoma and Neuroendocrine Carcinoma with Metastatic Potential


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ABSTRACT

Neuroendocrine (NE) cells may be involved not only in growth and differentiation of the normal prostate but also in carcinogenesis and progression of prostate adenocarcinoma (Pca), including development of androgen resistance. However, the exact pathophysiology of NE cells in Pca remains poorly understood. Here we describe a transgenic model of Pca with progressive NE differentiation. Seven lines of transgenic mice with the rat prostate-specific large probasin promoter linked to the SV40-large T antigen (Tag) that develop prostatic neoplasia have been established. In this study, one of the seven lines (12T-10) was characterized by examination of 52 mice aged from 2–12 months. With advancing age, low-grade prostatic intraepithelial neoplasia, high-grade prostatic intraepithelial neoplasia, microinvasion, invasive carcinoma, and poorly or undifferentiated carcinoma with NE differentiation appeared in the prostates in sequential order. Whereas Tag is expressed uniformly in prostate epithelium, only an increasing subset of cells in prostatic intraepithelial neoplasia showed NE differentiation by chromogranin immunostaining. Frankly invasive carcinoma developing subsequently showed occasional definitive glandular differentiation (adenocarcinoma) and particularly undifferentiated carcinoma with NE histological features similar to those observed in NE carcinomas in humans. The NE carcinomas occurred in the dorsolateral and ventral lobes and were generally androgen receptor negative. Twenty-one of 32 (66%) mice aged ≥6 months and 15 of 17 (88%) mice aged ≥9 months developed metastatic tumors, as confirmed by histology and/or Tag immunohistochemistry. Metastases occurred at the later time points, with metastasis to regional lymph nodes, liver, and lung being particularly common. Metastases showed histological features of NE differentiation, as confirmed by chromogranin immunostaining and electron microscopy. An athymic nude mouse that received a s.c. implant of a primary NE tumor developed Tag-positive metastatic tumors with similar NE differentiation. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry identified identical protein profiles between the primary NE tumor and lesions in the extraprostatic organs. Hence, in the 12T-10 large probasin promoter-Tag mouse, high-grade prostatic intraepithelial neoplasia develops progressively greater NE differentiation and progresses to invasive adenocarcinoma and NE carcinoma, with a high percentage of metastases. The predictable progression through these stages will allow testing of therapeutic interventions as well as possible further delineation of the role of NE cells in Pca progression.

INTRODUCTION

Prostate epithelium includes basal cells, luminal cells, and NE cells. Each cell type has a distinct phenotype and presumed function (1, 2). Recently, it has been speculated that NE cells are involved not only in growth and differentiation of the normal prostate but also in the pathogenesis of Pca (3–6). NE cells produce a number of bioactive secretory products such as biogenic amines and polypeptide growth factors, which may have growth-promoting paracrine effects on prostate cells (3–6). In addition, vascular endothelial growth factor and transforming growth factor-α secreted by NE cells may be involved in cancer growth and metastatic development through induction of angiogenesis (7). Furthermore, differentiation of androgen-independent NE cells has been postulated as one mechanism of development of hormone resistance of Pca (3–6). However, the exact roles of NE cells in Pca remain poorly understood. A transgenic animal model that develops precursor lesions, such as HGPIN, IC, and metastatic carcinoma with NE differentiation would be useful to further explore mechanisms involved in Pca progression, including the significance of NE cells.

To date, few efforts to generate transgenic models of NE Pca have been reported, and these have generally used non-prostate-specific promoters. Skalnik et al. (8) reported that 5′-flanking sequences of the human gp91-phox gene linked to the SV40 early region induced neuroblastoma (neuroectodermal tumor), with dense core secretory granules, in the prostate. However, some founders develop hematopoietic malignancies because the gp91-phox gene linked SV40 early region targets myelomonocytic cells (8). Perez-Stable et al. (9, 10) reported that one of the transgenic mouse lines containing the fetal globin promoter linked to the SV40 early region developed NE Pca. However, several other types of tumors including an adrenal tumor, a hibernoma, and a seminoma also developed in the transgenic mice. Recently, DiGiovanni et al. (11, 12) generated transgenic mice expressing human insulin-like growth factor-1 driven by a bovine keratin 5 promoter that is specifically targeted to the basal cell layer of multiple epithelia. Mice develop adenocarcinoma and NE carcinoma in the prostate as well as epidermal hyperplasia and hyperkeratosis in the skin (11, 12). However, the incidence of NE Pca was only 5% (2 of 38) in mice ≥6 months of age. Transgenic mice established with nucleotides −6500 to +34 of the mouse cryptdin-2 gene linked to the SV40 early region intended to target intestinal paneth cells surprisingly developed tumors in the prostate, which showed NE differentiation (13). The transgene was targeted to a subset of prostate epithelial cells with NE differentiation, and NE tumor nodules developed after prostatic intraepithelial neoplasia (PIN) and foci of microinvasion in...

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3 The abbreviations used are: NE, neuroendocrine; LBP, rat large probasin promoter; LGPIN, low-grade prostatic intraepithelial neoplasia; HGPIN, high-grade prostatic intraepithelial neoplasia; MI, microinvasive carcinoma; IC, invasive carcinoma; UC, undifferentiated carcinoma; Pca, prostate carcinoma; MALDI-TOF-MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; Tag, SV40 large T antigen; PCNA, proliferating cell nuclear antigen; AR, androgen receptor; CG, chromogranin A.
the prostate. At 24 weeks of age, 40% of the mice showed metastatic NE cancer in regional lymph nodes, liver, lung, and bone marrow (13). Although this promoter was subsequently shown to target an unrelated gene to the same cell population (13), its specificity for the prostate remains to be established.

The use of prostate-specific promoters is essential for limiting tumor development to the prostate and further assuring that metastatic tumors arise from prostate primaries versus other tumors that can occur with more widely expressed transgenes. The probasin promoter represents a useful prostate-specific promoter, specifically targeting transgene expression. Probasin is structurally related to the lipocalin protein superfamily (14). Although the ligand and the function of the protein remain unknown, probasin gene expression is prostate specific, regulated by androgen and zinc (15–17). Several transgenic mice that target transgene expression to the prostate have been generated using a small fragment of the probasin promoter (−426 to +28 bp) or a large fragment of the LPB promoter (−11,500 to +28 bp). Both probasin constructs confer androgen regulation and prostate-specific gene expression, although the level of transgene expression is greater with the LPB promoter (17–20). We have established seven transgenic mouse lines with a LPB promoter linked to SV40-large Tag (LPB-Tag), which contains a deletion in the early region to remove the small Tag (17). In a previous study, we demonstrated that the rate of prostate growth varies among the individual lines, likely due to the level of transgene expression. The rapidly growing LPB-Tag lines exhibited similar histopathological alterations, which preferentially start in the dorsolateral prostate, characterized by prominent glandular proliferation and cytologic atypia and generally accompanied by stromal hypercellularity. The large size of the prostate precludes maintaining some of these lines to later ages, and invasive disease is limited. In this study, we have characterized in detail the LPB-Tag line that shows the slowest prostate growth rate. We report that this line develops precursor lesions more analogous to human HGPIN, without associated prominent stromal hypercellularity (also more similar to human Pca). Furthermore, this line predictably develops IC with glandular differentiation (adenocarcinoma) as well as NE Pca that commonly metastasizes.

MALDI-TOF-MS is an ideal tool for the rapid analysis and characterization of proteins (21, 22). In recent years, it has been used in combination with several types of separation methods to characterize and sequence proteins from complex biological mixtures. Some studies have focused on the identification of peptides and proteins involved in cancer (23, 24). Identical protein profiles on MALDI-TOF-MS have focused on the identification of peptides and proteins involved in cancer (23, 24). Identical protein profiles on MALDI-TOF-MS between prostate primary and extraprostatic NE carcinoma show destructive overgrowth of normal prostate architecture essentially showing microscopic foci with cytological and histological features typical of NE differentiation. These included scant cytoplasm, with high nucleocytoplasmatic ratios, hyperchromatic or coarsely granular (“salt and pepper”) chromatin, nuclear molding, and rosette formation. Such foci were designated as UC (Fig. 1, F–H).

Immunohistochemistry. Immunostaining was performed on 5-μm-thick paraffin sections, which were deparaffinized and rehydrated, using standard techniques. For Tag, AR, and PCNA immunostaining, antigen retrieval was achieved with microwaving in 1 M urea for 30 min, and the slides were then left to cool in the urea solution for 1 h at room temperature. For CG immunostaining, antigen retrieval was achieved with microwaving in 0.01 M citrate buffer (pH 6.0) for 30 min, and for cytokeratin immunostaining, slides were pretreated with proteinase K digestion (Dako) for 10 min. Slides were rinsed with PBS and then placed in 3% hydrogen peroxide for 30 min to block endogenous peroxidase activity. After rinsing with PBS, the slides were placed in 1% blocking solution (Boehringer Mannheim, Mannheim, Germany) for at least 30 min to block nonspecific binding of antibody to the tissues. Sections were incubated with primary antibody overnight at 4°C. The following primary antibodies were used (with the indicated dilutions in PBS): (a) SV40 Tag, Ab-2 (Oncogene Research Products; 1:100); (b) AR, N-20 (Santa Cruz Biotechnol-
PC10 (Santa Cruz Biotechnology Inc.; and 1:50); (d) CG, bovine SP-1 (Diasorin Inc.; 1:1000). For Tag-immunostained sections, the same concentration of control mouse ascites fluid (Sigma Chemical Co.) was used as a negative control. After primary antibody incubation and washing with PBS, the slides were incubated with the appropriate secondary antibodies, either goat anti-mouse IgG (Sternberger Monoclonals Inc.; 1:300) or goat antirabbit IgG (Sternberger Monoclonals Inc.; 1:300) for 2 h at room temperature, rinsed with PBS, and then incubated in mouse or rabbit peroxidase-antiperoxidase complex (Sternberger Monoclonals Inc.; 1:500) for 1 h at room temperature. After soaking in 50 mM Tris-hydrochloride (pH 7.5), color development was performed with 3,3′-diaminobenzidine tetrahydrochloride (Dako). For PCNA, color development was performed after primary antibody incubation. Slides were counterstained with hematoxylin, dehydrated, and coverslipped.

**Electron Microscopic Examination.** For ultrastructural examination, tissues were fixed in EM grade 2% glutaraldehyde and embedded in Epon blocks, and thin sections were stained with uranyl acetate and lead citrate using standard conditions. Sections were examined using a Philips 301 transmission electron microscope.

**Establishment of Allograft Model.** A 3 × 3-mm block of the ventral lobe of a 12T-10 mouse (7408; 38 weeks old), which had a macroscopically obvious tumor, was implanted s.c. in the back of an 8-week-old athymic male mouse. Tumor growth was monitored, and the mouse was sacrificed at 18 weeks after inoculation, when the s.c. tumor showed exponential growth. The primary s.c. tumor and multiple organs were harvested for histological and immunohistochemical studies. The tumor was maintained by further s.c. passages.

**Mass Spectrometry.** Tissue samples from a nontransgenic CD1 mouse, a 12T-10 transgenic mouse (7408), and the allograft athymic mouse were immediately snap frozen after dissection. Details of the mass spectrometry procedure have been reported previously (21). In brief, thawed small tissue sections (2 × 2 × 2 mm) were blotted on the carbon-embedded polyethylene membrane for 5 min. After removing tissue sections, the membrane was allowed to dry completely. The blotted areas were washed thoroughly with water and allowed to dry again. Before mass spectrometry analyses, 1 μl of matrix (sinapinic acid; Sigma Chemical Co.) at 20 mg/ml in acetonitrile/0.1% trifluoroacetic acid in H2O (1:1, v/v) was deposited on the blotted areas and allowed to dry. Mass spectrometry analyses were performed using a DE-STR MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA). The instrument was operated in the linear mode under optimized delayed extraction conditions. An internal mass calibration was performed on each spectrum using the mass values of the previously measured singly and doubly charged ions of the α and β chain of hemoglobin using the singly charged molecular ions of bovine insulin (molecular weight, 5733.6; Sigma Chemical Co.) and horse heart cytochrome c (molecular weight, 12360.1; Sigma Chemical Co.).

**Statistical Analysis.** Statistical analyses were performed using the χ2 test and Spearman’s rank correlation test.

**RESULTS**

**Histopathological Progression of Prostatic Neoplasia in the 12T-10 LPB-Tag Line.** Epithelial proliferation with mild nuclear atypia, compatible with LGPIN, was observed in most of lobes of 2–5-month-old mice (Fig. 1A; Tables 1 and 2). The degree of epithelial proliferation, nuclear stratification, and cytological atypia progressed quickly, such that HGPIN was observed in the prostates of...
2-month-old mice and occupied progressively greater foci, becoming the dominant abnormality with advancing age (Fig. 1B). LGPIN was essentially no longer identified in mice ≥6 months of age. HGPIN was present uniformly throughout preexisting glands and ducts. In contrast to other LPB-Tag lines (17), in which there is marked glandular proliferation with expanded lobules of arborizing large and small gland profiles quite architecturally distinct from wild-type mice, the cytologically atypical epithelial proliferation in 12T-10 mice appeared to be confined to gland profiles generally similar to those of wild-type mice. As such, the lesion designated as HGPIN was architecturally more analogous to that in human prostate (17, 19). Likewise, also not generally present was marked stromal proliferation, which is not a feature of human HGPIN or Pca lines or some other reported transgenic mouse models

### Table 1 Pathology of prostate and metastasis of 12T-10 mice

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<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>HGPIN, IC</td>
<td>RLN, liver, spleen, kidney</td>
</tr>
<tr>
<td></td>
<td>488</td>
<td>HGPIN, MI, IC</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>RLN, liver, lung</td>
</tr>
<tr>
<td></td>
<td>489</td>
<td>HGPIN, MI, IC</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>RLN, liver, lung</td>
</tr>
<tr>
<td></td>
<td>848</td>
<td>HGPIN, MI, IC</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>RLN, liver, lung</td>
</tr>
<tr>
<td></td>
<td>849</td>
<td>HGPIN, MI, IC</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>RLN, liver, lung</td>
</tr>
<tr>
<td></td>
<td>970</td>
<td>HGPIN, MI, IC</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>RLN, liver, spleen, bone&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>973</td>
<td>HGPIN, MI, IC</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2372</td>
<td>HGPIN, MI, IC</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>HGPIN, MI</td>
<td>UC</td>
</tr>
</tbody>
</table>

<sup>a</sup> MI, microinvasion; IC, invasive carcinoma without histological features of glandular or neuroendocrine differentiation; ICad, invasive carcinoma with glandular differentiation; ICad, invasive carcinoma with neuroendocrine differentiation; ICad, invasive carcinoma with glandular and neuroendocrine differentiation; RLN, regional lymph nodes; MLN, submandibular lymph nodes; NA, not available.

<sup>b</sup> Bone metastases by Tag-positive immunostaining.

<sup>c</sup> The ventral lobe which had nodular neuroendocrine carcinoma was transplanted in an athymic male mouse to establish an allograft model and provided for mass spectrometry analysis.

<sup>d</sup> Regional lymph nodes and liver that had Tag-positive lesions were provided for mass spectrometry analysis.
ably to IC, being uniformly and diffusely present in prostate sections that developed MI and IC. MI and IC (as defined in “Materials and Methods”) were identified in mice 4 and 6 months of age, respectively (Fig. 1, C–F). As indicated in “Materials and Methods,” the uniform presence of HGPIN and scant stroma for spatial separation of single cells or small acinar structures from immediately adjacent ducts/glands with HGPIN makes recognition of MI difficult. The designation of such lesions in the current study as MI was supported by the fact that metastases were occasionally detected (see below) in animals in which only such lesions (as opposed to obvious destructive invasion) were present in prostate sections.

More definitive foci of invasion (IC or UC) were present in 40% of mice 6–7 months of age, 67% of mice 8–10 months of age, and 100% of mice 11–12 months of age (Tables 1 and 2). Small (<2 mm) but definitive foci of IC were composed of nests with definitive glandular differentiation (adenocarcinoma; 54%; Fig. 1D); nests with cytological and architectural features of NE differentiation, including scant cytoplasm, nuclear molding, and rosette formation (19%; Fig. 1E); and small nests of malignant epithelial cells with varying amounts of cytoplasm and no obvious light microscopic glandular or NE differentiation (19%). Rosette formation in NE tumors can be difficult to distinguish from more usual glandular differentiation, and NE carcinomas in various organs in humans can show glandular differentiation. In several small invasive foci, features of both glandular and NE differentiation were noted (8%). Both types of invasive tumor differentiation (adenocarcinoma and NE) always occurred in the setting of uniform HGPIN. Further evidence of progression of invasive foci to more definitive NE differentiation was that larger invasive tumors (≥2 mm; generally with destruction of adjacent parenchyma) all showed unequivocal light microscopic features of NE differentiation. These tumors, designated UC, were not detected in mice ≤7 months of age and were present in 20% of 8-month-old mice, and 29% of ≥9-month-old mice (Fig. 1, F–H).

### Table 2 Relationship between age group and histology of the 12T-10 prostates

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>No. of mice</th>
<th>LGPIN</th>
<th>HGPIN</th>
<th>MI</th>
<th>IC</th>
<th>UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
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<td>5 (100)</td>
<td>5 (100)</td>
<td>1 (20)</td>
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<td>5</td>
<td>2 (40)</td>
<td>5 (100)</td>
<td>2 (40)</td>
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<tr>
<td>6</td>
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<td>0</td>
<td>5 (100)</td>
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<td>2 (40)</td>
<td>0</td>
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<td>5</td>
<td>0</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>2 (40)</td>
<td>0</td>
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<tr>
<td>8</td>
<td>5</td>
<td>0</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>3 (60)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>9–10</td>
<td>7</td>
<td>0</td>
<td>7 (100)</td>
<td>7 (100)</td>
<td>4 (57)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>11–12</td>
<td>10</td>
<td>0</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>9 (90)</td>
<td>3 (30)</td>
</tr>
</tbody>
</table>

Fig. 2. Immunohistochemistry in HGPIN and IC of 12T-10 transgenic mice. A, B, and D stain with CG in the dorsal lobe of a 10-month-old mouse (A) and the dorsolateral lobe of a 12-month-old mouse (B and D). C and E, large Tag (C, serial section of B) and AR (E, serial section of D) in HGPIN of a 12-month-old mouse. F, CG in IC with NE differentiation in the dorsolateral lobe of a 12-month-old mouse. G, absent CG (compared to HGPIN in A, B, and D) immunostaining in focus of IC with glandular differentiation in the dorsolateral lobe of an 11-month-old mouse.
NEUROENDOCRINE CARCINOMA IN LPB-Tag TRANSGENIC MICE

Incidence of Regional and Systemic Metastases in the 12T-10 Line. Metastases were detected by routine histology on sampled nodes and organs (see “Materials and Methods”), supplemented by Tag immunohistochemistry to increase sensitivity of detection of micrometastases. Metastatic carcinoma occurred with increasing frequency with age, paralleling the extent of definitive IC and especially the appearance of the poorly differentiated NE carcinoma in the prostate (Table 1). Two- to 5-month-old mice did not have detectable metastases, whereas metastases were present in 6 of 15 (40%) of mice 6–8 months of age and 15 of 17 (88%) mice of 9–12 months of age ($P < 0.001$, $\chi^2$ test). The most common sites of metastases were regional lymph nodes, liver, and lung. These lesions were histologically similar to the UC in the prostate and were composed of large sheets of cells with NE differentiation, including scant cytoplasm, nuclear molding, and rosette formation. NE differentiation was confirmed by focal chromogranin A immunostaining and rare dense core neurosecretory granules (Fig. 4A). As with the histologically identical UC tumors in the prostate, cytokeratin immunostaining was noted but was weak, and AR immunostaining was largely negative, with only focal weak staining. Definitive adenocarcinoma morphology, as noted in smaller invasive lesions in the prostate, was not detected in metastatic foci.

Metastatic lesions were strongly Tag immunopositive (Fig. 4B). Of the 21 mice with Tag-immunopositive metastases, 15 (71%), 14 (67%), and 13 (62%) had Tag-positive lesions in the regional lymph nodes, liver, and lung, respectively (Table 1). Liver metastases in particular were occasionally bulky, measuring up to 1 cm. The average number of extraprostatic sites with Tag-positive metastatic lesions per mouse increased with advancing age as follows: 0, 0.4, 1.0, 2.3, and 2.9 in mice 2–5, 6–7, 8, 9–10, and 11–12 months of age, respectively ($P < 0.001$, Spearman’s rank correlation test). Tiny Tag-positive lesions in the bone marrow were found in 3 of 44 (6.8%) mice examined, although cytologically malignant corresponding foci were not identified in serial sections. Three mice had Tag-positive cells in the bulbourethral glands. Two of three mice (7408 and 7603; both 9–10 months of age) had Tag-positive UC with NE differentiation in the bulbourethral glands, whereas the remaining mouse (970; 11–12 months of age) had Tag-positive epithelial cells within the bulbourethral acini without any cytological or structural abnormality. Both of the mice with UC in the bulbourethral glands had lymph node and visceral metastases with similar morphology. One mouse had UC in the ventral lobe, and the other had IC with NE differentiation in the anterior lobe. Whether the UC tumors in the bulbourethral glands represented metastases or distinct primaries is not certain. Tag immunostaining was detected in benign bulbourethral glands in one mouse, and others have observed transgene expression and tumor development in bulbourethral glands with other supposedly prostate-specific promoters (26), which suggests that pathology in these foci could result from transgene expression at this site. Mice with tumors in the bulbourethral glands in this series had tumors in the prostate, and similar systemic metastases were commonly observed in mice with such tumors in the prostate but no pathology in the bulbourethral glands.
glands; these observations suggest that the prostate is the source for metastatic disease even in those animals with a histologically similar tumor at this other genitourinary site.

**Correlation of Metastases with Pathology of the Prostate.** Mice that only had LGPIN and/or HGPIN on examined sections of prostate never had metastases in extraprostatic organs (Table 3). On the other hand, 33% of mice with MI, 69% of mice with IC, and 100% of mice with UC had metastases. Thus, the incidence of metastatic tumors paralleled the progression of neoplasia in the prostate and increased with the extent of local invasion, such that the existence of IC and UC clearly correlated with high metastatic potential in older mice.

**Allograft Model of NE Tumor.** To further confirm the metastatic potential of the UC developing in the 12T-10 prostate, portions of the grossly abnormal ventral lobe of a 38-week-old 12T-10 mouse (7408) that had a histologically confirmed UC tumor were transplanted s.c. into an athymic male mouse. A histologically identical NE carcinoma grew at the primary transplant site, and the mouse developed metastatic disease even in those animals with a histologically similar tumor at this other genitourinary site.

**Mass Spectrometry Analysis.** Mass spectrometric analysis of the UC-containing ventral lobe of a 38-week-old 12T-10 mouse (7408) showed distinct and specific protein profiles compared to the ventral lobe of a nontransgenic CD1 mouse (Fig. 5). For example, the signals observed from the ventral lobe of the nontransgenic CD1 mouse (Fig. 5). For example, the signals observed from the ventral lobe of the nontransgenic CD1 mouse (7408) contained at least five different ions at mass 11,265, 11,307, 11,349, 11,391, and 11,433 Da. The signal distribution was Gaussian shaped, suggesting multiple random acetylation. Signal a and signal e displayed two major peaks at mass 12,132 Da/12,167 Da and 13,777 Da/13,804 Da, respectively. Signal d contained three distinct mass peaks at 14,005, 14,047, and 14,089 Da. Signal e was located between the a and the b chains of hemoglobin. This signal centered on 15,355 Da and was broad and unresolved, indicating the presence of several different proteins or protein isoforms within a close mass range. Based on the protein database searched (SwissProt) using the measured molecular masses, a tentative identification of the signals a through e could be made. The signal mass 11,265 Da could be identified as the histone H4, and the following signals at 13,307, 13,349, 13,391, and 13,433 Da were consistent with multiple histone H4 acetylations [up to five successive acetylations have been reported on the histone H4 (27)]. The signal observed at 13,804 Da (from the c signal cluster) could be identified as histone H2B1. The signals observed at 14,005 and 14,047 Da (from the d signal cluster) were consistent with histone H2A.2 and its diacetylated forms, respectively. The signal detected at 14,089 Da (from the d signal cluster) could be attributed to histone H2A.1. Finally, based on the observation of several different histones in the protein profile, the broad signal observed around 15,355 Da (signal e) could be assigned to the histone H3. No tentative identifications were made for the signals in the b cluster or for the signal at 13,777 Da from the c cluster.

**Table 3. Relationship between metastatic development and histology of the 12T-10**

<table>
<thead>
<tr>
<th>Histology of the prostate</th>
<th>Metastasis (-)</th>
<th>Metastasis (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGPIN/HGPIN</td>
<td>18 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>MI</td>
<td>8 (67)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>IC</td>
<td>5 (31)</td>
<td>11 (69)</td>
</tr>
<tr>
<td>UC</td>
<td>0 (0)</td>
<td>6 (100)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>31</strong></td>
<td><strong>21</strong></td>
</tr>
</tbody>
</table>

lobes of the 12T-10 mouse showed profiles similar to those of the ventral lobe (Fig. 6). These lobes contained HGPIN, MI, and IC (also present in the ventral lobe) suggesting that these proteins may not be unique to the larger invasive undifferentiated or NE carcinoma but may be shared by a population of cells common to all of the precursor and invasive lesions. Whether this relates specifically to NE differentiation is not clear. Furthermore, the anterior, lateral, and dorsal lobe peak intensities were lower than those in the ventral lobe, suggesting a possible increased expression of the signal source proteins with progression (Fig. 6). Samples from regional lymph nodes and liver of the 12T-10 mouse with only metastatic NE carcinoma showed exactly the same protein profiles as the ventral lobe. In addition, samples from a s.c. inoculated NE carcinoma from the athymic nude mouse and its liver metastasis also had the same protein profiles as the NE carcinoma in the ventral lobe of the 12T-10 transgenic line (Fig. 7).

**DISCUSSION**

There is definite need for sophisticated and validated transgenic mouse models of human Pca. Having animal models that progress predictably through precursor lesions, analogous to human HGPIN, to early and more established IC and eventually to higher grade lesions with metastatic potential would parallel defined stages in human Pca. If such models are established by genetic manipulations that result in additional molecular alterations with lesion progression that are also implicated in progression of human Pca, they will be extremely useful. Such changes would allow for testing of mechanistically relevant interventions at different stages of disease evolution, with clear end points for assessing efficacy. These include primary chemoprevention at the precursor stage or secondary pharmacological intervention once invasion has occurred to attempt to delay progression and prevent more extensive and metastatic disease, neither of which desired treatment strategies currently exists for human Pca. Finally, such models may allow for testing treatments in advanced, metastatic disease, which have lost androgen sensitivity. With these issues in mind, the animal model presented in the current study represents a distinct advance in transgenic models of Pca.

The SV40 early region has been used in transgenic constructs with other promoters, some of which are designed to be prostate specific (19, 28), some of which unexpectedly targeted the prostate (13), and some of which result in transgene expression in other organs (8–10). The LPB is androgen dependent and prostate specific (16, 20), desirable properties in the establishment of a transgenic Pca model. The small Tag was inactivated in the constructs used to generate the LPB-Tag lines, including 12T-10 (17), thus narrowing the possible molecular mechanisms of transformation. The large Tag sequesters and inactivates p53 and retinoblastoma, altering cell cycle regulation and possibly leading to genetic instability (29, 30). Although p53 mutations have been accepted as present in a subset of human Pca...
hemoglobin, respectively. icaically similar to that in human because the cytologically atypical reported herein as HGPIN in the 12T-10 mouse is more morpholog- 
glands, as in human HGPIN (25, 38). In these two respects, the lesion 
within otherwise architecturally unaltered preexisting ducts and 
the occurrence of the epithelial proliferation (nuclear stratification) 
basement membranes and lacking metastatic potential, as opposed to 
the noninvasive stages of these prostate neoplasias, there appears to 
common prostatic epithelial neoplasia in the human. Furthermore, in 
liferations. This stromal proliferation is not a feature of the usual or 
with atypia) accompanying the cytologically atypical epithelial pro-

promoter and the SV40 early region (19) and previously characterized 
invasive or metastatic tumor in some models lack morphological 
Furthermore, the described precursor lesions preceding unequivocal 
molecular alterations to IC and a condition having a significant 
HGPIN on morphological grounds (35) do not appear to progress and 
progression to invasive tumor (36, 37). However, the development of metastases in the animal model allowed us 
to “work backward” with regard to histological features of early invasion 
in the prostate. Morphologically similar microinvasion into the thin rim of 
surrounding stroma was observed in prostates of mice created with the 
SV40 early region on the cryptidin-2 promoter (13) and under the control 
of the rat prostatic steroid-binding protein [C3(1)] gene (42). As in 
the current series, the development of greater degrees of invasion in both 
these models and metastatic disease in the former supports such lesions 
as truly invasive.

The invasive foci in the 12T-10 mouse showed both glandular 
differentiation and NE differentiation. Invasive adenocarcinoma, 
based on true extension into periprostatic fat and local tissues, has 
been reported recently in mice overexpressing human insulin-like 
growth factor I targeted to basal epithelium with the bovine keratin 5 
promoter (12). These mice did not develop metastases. In addition, 
similar to other previously reported models, the lack of utilization of 
prostate-specific promoters may lead to neoplasia in other organs, 
potentially complicating interpretation in those models that do event-
ually progress to systemic metastases either spontaneously or by 
cross-breeding with other transgenic mice. With larger invasive foci 
and with metastatic disease in the 12T-10 mouse, primary and meta-
static tumors showed cytological and histological features typical of 
NE differentiation, which was confirmed immunophenotypically and 
ultrastructurally. The incidence of metastases increased with age and 
did not occur in mice <6 months of age but was increasingly common 
thereafter and found in 66% of mice ≥9 months of age and 88% of 
mice ≥9 months of age. Furthermore, metastatic development clearly 
correlated with the existence of IC and especially of UC in the 
prostate. Metastases occurred in both regional lymph nodes and dis-
tant organs. The results indicate that there was both lymphatic and 
hematogenous metastatic spread, similar to that seen in human Pca 
(43). Hematogenous visceral metastases (e.g., liver and lung) are 

5 S. B. Shappell, unpublished observation.
showed the clusters of signals detection of a large amount of histones may represent active prolif-
trated, and their protein content was accessible to the blotting mem-
ratios of NE cancer cells. When the tissue sections were prepared,
spectrometry. This may be related to the high nuclear:cytoplasmic
proteins, were observed in NE carcinoma in high abundance by mass

distinct from those of wild-type prostate. Histones, typically nuclear
proteins, were observed in NE carcinoma in high abundance by mass
spectrometry. This may be related to the high nuclear:cytoplasmic
ratios of NE cancer cells. When the tissue sections were prepared,
invariably, a nonnegligible percentage of the cell nuclei were rupt-
tured, and their protein content was accessible to the blotting mem-
brane. Although histones themselves are not specific for NE cancer,
detection of a large amount of histones may represent active prolifer-
ation through high transcription of NE cancer cells because only
12T-10 prostates and those metastases containing NE cancer cells
showed the clusters of signals a through e. The unidentified signals in the
b cluster and the signal at 13,777 Da from the c cluster may imply
NE cancer-specific markers of the 12T-10 mice. Thus, mass spectrom-
etric analysis may become one strategy to characterize possibly
changing protein expression profiles during tumor progression and to
help establish sites of primary origin for metastatic tumors.

Recently, interest has focused on the role of NE differentiation in
PCA (3–6). It has been reported that 30–100% of human PCA have
focal or extensive NE differentiation (3, 6). Although NE cells per se
are thought to be postmitotic cells (45, 46), it has been demonstrated
that adenocarcinoma cells near NE cells express the proliferation
marker Ki-67 and the apoptosis-inhibiting proto-oncogene bcl-2 (45, 47).
Thus, NE cells may influence the behavior of adjacent adenocar-
cinoma cells through a paracrine mechanism. However, it is still
controversial whether NE differentiation in PCA correlates with poorer
prognosis (3–6). It has also been reported that the majority of prostatic
NE cells do not express detectable nuclear AR (48, 49). Although
Nakada et al. (50) reported that most NE cells have AR expression,
they noted that a subpopulation of AR-negative NE cells is more
prominent in PCA than in benign prostatic tissues. Casella et al. (51)
reported a significant increase in the frequency and density of NE
differentiation in specimens of hormone-refractory PCA compared to
those obtained before hormonal treatment from the same patients.
Thus, expansion of an androgen independent NE cell population in
PCA is one possible mechanism for the development of androgen
resistance in advanced PCA. In the current study, development of
larger primary and metastatic tumors with NE differentiation was
accompanied by reduced AR immunostaining. Surprisingly, all the
12T-10 tumors continue to express the Tag gene, which is under the
regulation of the androgen-dependent probasin promoter. If similar
mechanisms are ultimately determined to be responsible for loss of
androgen dependence in advanced human PCA and the 12T-10 mouse,
this model will be extremely useful for characterizing the role of NE
cells in evolving androgen resistance and for testing therapeutics for
advanced androgen-insensitive malignancy.

Small cell carcinoma may be present at initial diagnosis or may
develop subsequently in patients with a previous history of more usual
acinar PCA. Patients with small cell carcinoma of the prostate have
a poor prognosis, and the tumor does not appear to respond to hormonal
therapy (44, 52, 53). Although small cell carcinoma is composed of
NE cells, these cells show striking proliferative activity (53). Thus, the
characteristics of NE cells in small cell carcinoma are very different
from postmitotic NE cells that may be observed in prostate adenocar-
cinoma. Similarly, advanced tumors in 12T-10 mice showing pure
cytological NE differentiation were extremely active mitotically.
Small cell carcinoma may originate from pluripotent malignant cells
that differentiate to both acinar-forming cells and NE cells (44, 53).
The relationship of small cell carcinoma to more usual acinar PCA
and the mechanism of progression potentially accompanying exogenous
hormonal manipulation are poorly understood (3). Because existing in vitro and in vivo models from human small cell carcinoma have been
established from metastatic lesions or recurrent lesions in patients that
fail hormonal therapy (54, 55), they preclude examining the histogen-
esis and natural history required for the development of small cell
carcinoma. Therefore, an animal model that spontaneously develops
NE prostate cancer would be useful to study the evolution of this
cancer.

By immunostaining for chromogranin A and/or neuron-specific
enolase, there are only 6–7 NE cells/10 high-power fields of normal
adult prostatic acini (56). Our study similarly demonstrates that the
NE cell population is quite rare in the prostatic acini of adult non-
transgenic (normal) mice. On the other hand, prostates of 12T-10 mice
≥5 months of age showed an increasing population of NE cells within
HGPIN that also stained for Tag. Because Tag is expressed in essen-
tially all prostate epithelial cells at early time points (17) at which
little or no NE differentiation is present, it would appear that an
increasing subset of Tag-positive cells is developing NE differentia-
tion as PIN progresses in the 12T-10 line. It is likely that the prolif-
erating compartment in this evolving lesion includes NE cells that
give rise to invasive and eventually metastatic tumors with definitive
cytological and histological features of NE carcinoma. The apparent
mechanism of progression of Tag-positive epithelial cells to neoplas-
cic cells with NE differentiation is in contrast to the transformation
achieved by the use of the cryptdin-2 promoter linked to the SV40
early region, in which for unknown reasons the transgene was ex-
pressed in only the small subset of prostate epithelium that already
showed NE differentiation (13). It is not clear if acinar forming
invasive foci in the 12T-10 mouse arise from HGPIN cells with or
without NE differentiation. Although definitive invasive foci with
acinar formation were noted to be CG negative, they were also
occasionally seen in association with histologically typical NE carci-
noma. The latter lesions also show only faint CG immunostaining
perhaps similar to reduced neurosecretory granules and CG immuno-
staining in progressively higher grade human NE carcinomas.
Whether the same molecular alterations in the HGPIN lesions are
responsible for developing invasive adenocarcinoma or IC with NE
differentiation remains to be discerned. However, based on size and
incidence and histological appearance of metastases, it appears that
invasive lesions progress quickly to NE carcinoma in this model,
perhaps in ways similar to the more protracted progression in human
PCA.

REFERENCES
1. Bonkhoff, H., and Remberger, K. Differentiation pathways and histogenetic aspects of


16. Rennie, P. S., Bruchovsky, N., Leco, K. J., Sheppard, P. C., McQueen, S. A., Cheng, 


12. DiGiovanni, J., Kiguchi, K., Frijhoff, A., Wilker, E., Bol, D. K., Beltran, L., Moats, 

11. DiGiovanni, J., Bol, D. K., Wilker, E., Beltran, L., Carbajal, S., Moats, S., Ramirez, 


5. di Sant'Agnese, P. A. Neuroendocrine differentiation in prostatic carcinoma: an


A Probasin-Large T Antigen Transgenic Mouse Line Develops Prostate Adenocarcinoma and Neuroendocrine Carcinoma with Metastatic Potential


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