Prostatic intraepithelial neoplasia in mice expressing an androgen receptor transgene in prostate epithelium

Michael Stanbrough*, Irwin Leav†, Paul W. L. Kwan†, Glenn J. Bubley*, and Steven P. Balk*‡

*Cancer Biology Program, Hematology–Oncology Division, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215; and †Department of Pathology and Biomedical Sciences, Tufts University Schools of Medicine and Veterinary Medicine, Boston, MA 02111

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Prostate cancer (PCa) is an androgen dependent disease that can be treated by androgen ablation therapy, and clinical trials are under way to prevent PCa through the reduction of androgen receptor (AR) activity. However, there are no animal models of AR-mediated prostatic neoplasia, and it remains unclear whether the AR is a positive or negative regulator of cell growth in normal prostate secretory epithelium. To assess the direct effects of the AR in prostate epithelium, a murine AR transgene regulated by the rat probasin promoter (Pb) was used to generate transgenic mice expressing increased levels of AR protein in prostate secretory epithelium. The prostates in younger (<1 year) Pb-mAR transgenic mice were histologically normal, but Ki-67 immunostaining revealed marked increases in epithelial proliferation in ventral prostate and dorsolateral prostate. Older (>1 year) transgenic mice developed focal areas of intraepithelial neoplasia strongly resembling human high-grade prostatic intraepithelial neoplasia (PIN), a precursor to PCa. These results demonstrate that the AR is a positive regulator of cell growth in normal prostate epithelium and provide a model system of AR-stimulated PIN that can be used for assessing preventative hormonal therapies and for identifying secondary transforming events relevant to human PCa.

Prostate cancer (PCa) is an androgen-dependent disease and a leading cause of cancer morbidity and mortality in men (1, 2). The androgen receptor (AR) is a steroid hormone receptor member of the larger nuclear receptor superfamily of ligand-activated transcription factors (3–5). The vast majority of prostate cancers express the AR, are androgen dependent for their growth, and initially respond to androgen ablation therapy (6, 7). PCa that recurs after androgen ablation therapy also expresses the AR, and AR gene amplification and mutations contribute to disease progression (8–12). Epidemiological data further suggest a role for increased AR activity in stimulating PCa development, as higher testosterone levels and lower levels of sex steroid binding globulin are associated with an increased risk of PCa (13, 14). Increased PCa risk is also associated with ARs containing shorter polyglutamine (CAG) repeats in exon 1 (15–20), which are transcriptionally more active or may be more highly expressed *in vivo* (21–23). Taken together, these observations support a role for the AR in PCa development and progression.

AR protein is highly expressed in normal prostate by secretory epithelial cells and to a lesser extent by a subset of stromal smooth muscle cells (24, 25). However, in contrast to the direct stimulation of PCa growth by androgens, *in vitro* studies have shown that androgens do not stimulate the growth of normal prostate epithelial cells (26–28). Moreover, the AR can induce cell cycle arrest or apoptosis when transfected into AR-negative PCa cell lines or when stimulated in PCa cell lines adapted to grow at low androgen levels (29–34). AR expressed by prostate stromal cells can also induce the production of growth factors that indirectly stimulate the development and growth of prostate epithelial cells (35–37). Therefore, whether the AR can stimulate prostate epithelial cell growth *in vivo* and the relative role of AR in epithelium versus stroma are uncertain.

The important role of the AR in PCa has stimulated efforts to model PCa in animals through administration of exogenous androgens. Chronic administration of high dose testosterone to some strains of rats has been reported to cause prostatic neoplasms, but these appear to originate in seminal vesicle (38). Combined treatments with testosterone, and a mutagen (39) or estradiol (40, 41), are generally required to develop PCa in rat models, and androgen-induced PCa in mice has not been reported. Further insight into the role of the AR in regulating prostate growth was obtained from the classic studies of Bruchovsky *et al.* on castrated rats (42). They demonstrated that AR levels decreased immediately after castration and that readministration of androgen increased epithelial AR levels, with a concomitant dramatic proliferative effect on prostate epithelium. However, AR levels and the rate of DNA synthesis declined to normal levels once the differentiated epithelial cell population achieved precastration levels, and continued androgen supplementation did not increase cell numbers. These results demonstrate that androgen-mediated cell proliferation and AR levels are tightly controlled in the prostate and suggest that the respective regulatory processes may be coupled. Indeed, the AR can positively and negatively regulate AR mRNA levels via response elements in the promoter and coding region (43, 44).

Based on these observations, we speculated that AR levels in prostate epithelium might be an important factor regulating proliferation and that increased AR expression might result in a higher proliferative rate and increased PCa risk. This hypothesis was tested by selectively augmenting AR expression in prostate epithelium, by using a fragment (−426 to +28) of the rat probasin (Pb) promoter to target a murine AR (mAR) transgene to prostate secretory epithelium in sexually mature mice (45, 46). The Pb-mAR transgene was transcribed specifically in prostate and caused increased AR expression in the epithelium, but not the stroma. A marked increase in proliferation was demonstrated in secretory epithelium in histologically normal ventral prostate (VP) and dorsolateral prostate (DLP) glands from all Pb-mAR transgenic mice when compared with wild-type littermate controls. Aged Pb-mAR transgenic mice developed moderate to severe intraepithelial dysplasias with the histological

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Abbreviations: PCa, prostate cancer; AR, androgen receptor; mAR, murine AR; Pb, probasin; PIN, prostatic intraepithelial neoplasia; VP, ventral prostate; DLP, dorsolateral prostate; RT-PCR, reverse transcription–PCR; PAS, periodic acid/Schiff.

‡To whom reprint requests should be addressed at: Hematology–Oncology Division, Beth Israel Deaconess Medical Center, HIM Building, Room 1050, 330 Brookline Avenue, Boston, MA 02215. E-mail: sbalk@caregroup.harvard.edu.

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features of human high-grade prostatic intraepithelial neoplasia (PIN), believed to be a precursor to PCa (47). These results support a role for increased AR activity in stimulating epithelial proliferation and provide a murine model of AR-mediated prostatic neoplasia.

Materials and Methods

Pb-mAR Transgene Construction. Mouse AR has only an eight-residue CAG (glutamine) repeat in exon 1 (48), so no effort was made to further decrease this repeat. A promoterless 3.6-kb *Sal*I fragment containing the complete mAR cDNA and a rabbit β-globin polyadenylation site from $pmAR_0$ (48) was subcloned into the *Sal*I site of pBlueScriptSK+ (pSKmAR) (Stratagene). The −426 to +28 probasin promoter was excised from pBH500 (49) as a *Hin*dIII-*Bam*HI fragment and was blunt ligated to *Xho*I-cut pSKmAR to give the probasin-mouse androgen receptor vector Pb-mAR (pMS501–9F). The 4.0-kb Pb-mAR was excised with *Kpn*I and *Xba*I digestion, and pronuclear microinjection was performed at the Beth Israel Deaconess Medical Center Transgenic Facility in the FVB strain. Transgenic mice were identified by PCR of tail DNA with the use of probasin promoter 5′-AATCCACAGTTCAGGTTCAATG-3′ (−384 to −363) and mAR 5′-AGCTGAGTCATCCTGATCTG-3′ (534 to 515) or mAR 5′-CTCCTCGATAGGTCTTGGATG-3′ (193 to 173). All procedures were in accordance with institutional protocols.

Histology. Prostates were harvested by removing the bladder–urethra–prostate complex in one piece, with or without attached seminal vesicles, placed directly in 50 ml of neutral buffered 10% formalin at room temperature for 4 to 6 h, and machine dehydrated to paraffin immediately after fixation. Specimens were embedded to obtain sagittal sections of the urethra such that VP and DLP were in the same plane.

Immunohistology. Fresh 5-μm paraffin sections were baked at 60°C for 1 h just before processing, then brought to water and antigen retrieved by boiling in 10 mM sodium citrate (pH 6.2) for 20 min and cooling for 2 h. Blocking steps were with 1× PowerBlock and 1× Protein Block, each for 10 min at 25°C (BioGenex Laboratories, San Ramon, CA). Ab was diluted in Common Ab Diluent (BioGenex Laboratories) at 1:50 for the Ki-67 Ab (Immunotech, Westbrook, ME) and at 1:50 for AR Ab (Upstate Biotechnology, Lake Placid, NY). Primary Abs were incubated overnight at 5° followed by a biotinylated anti-IgG linking Ab (Multilink; BioGenex Laboratories) at 1:50 for 30 min at 25°C, and then a horseradish peroxidase–streptavidin conjugate (BioGenex) at 1:50 for 30 min at 25°C. Color was developed with 50 mM Tris·Cl (pH 7.6), 0.06% hydrogen peroxide, and 0.5 mg/ml diaminobenzidine for 1 min to 5 min, and nuclei were counterstained with hematoxylin.

Reverse Transcription–PCR (RT-PCR). Anterior prostate, DLP, and VP were dissected and processed separately and were substantially freed of extraneous fatty and connective tissues. RNA was extracted in RNAzol B (Tel-Test, Friendswood, TX), and reverse transcription was with 1 μg of RNA and an oligo-dT primer. PCR was with a common 5′ sense primer in exon 7 of the mAR: (2594–2613) 5′-AAGAAAGAATCCCACATCC-3′ (no. 5722), in conjunction with antisense primers on distinct exons to prevent amplification from any contaminating genomic DNA. The endogenous mAR was detected with an antisense exon 8 primer in the 3′ untranslated region, 5′-CAGAGAAGTAGTGCAGAGTT-3′ (no. 6417). The transgene transcript was detected with an antisense primer in the 3′ exon encoding the rabbit β-globin polyadenylation signal, 5′-CCACACCAGCCACCACCTTC-3′ (no. 5711).

AR Immunoblotting. Frozen VP and DLP samples were ground with a micropestle in 1% SDS, heated to 100°C for 15 min, and centrifuged at 45,000 rpm for 25 min in a Beckman TLA 45 rotor. The protein content of the lysates was assayed with bicinchoninic acid reagent (Pierce), and 40 μg was run reduced on SDS/PAGE and transferred to nitrocellulose membranes. Blots were blocked in 5% nonfat dry milk, then incubated with AR Abs (Santa Cruz Biotechnology SC-816 and SC-815, each at 1:2,000) overnight at 5°C. Washed blots were then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Promega) at 1:5,000, washed extensively, and developed with Renaissance chemiluminescence reagent (NEN).

Terminal Deoxynucleotidyltransferase-Mediated UTP End-Labeling Assays. Formalin-fixed paraffin sections were brought to water and antigen retrieved as above. Terminal deoxynucleotidyltransferase-mediated UTP end labeling assays were carried out with an *In Situ* Cell Death Detection Kit, TMR Red (Roche Pharmaceuticals, Nutley, NJ), according to the manufacturer's directions. Slides were mounted with Fluoromount-G (Southern Biotechnology Associates).

Results

Generation of Transgenic Mice. The rat probasin promoter (−426 to +28), which is positively regulated by the AR and expressed specifically in the prostate epithelium of sexually mature mice (45, 46), was used to target a mAR transgene to prostate. Transgenic mice were generated by pronuclear injection in FVB mice, and founders were identified that transmitted the Pb-mAR transgene to their progeny. RT-PCR was used to identify founder lines expressing the mAR transgene in prostate. Wild-type and transgenic AR cDNA were coamplified with the use of a common exon 7 sense primer and antisense primers on distinct exons specific to the unique 3′ untranslated regions of the endogenous and transgenic ARs, yielding a slightly larger product from the transgenic AR. Transgene expression was detected in prostates from three of four founder lines examined (Fig. 1*A* and data not shown).

Consistent with previous studies of this probasin promoter, the transgene was expressed in prostate (anterior prostate, VP, and DLP) but was not detected in kidney, liver, or testis (Fig. 1*B*). Whereas the Pb promoter is targeted selectively to prostate secretory epithelium, the endogenous AR transcript is also expressed in prostate by stromal and basal cells (24, 25). Therefore, the RT-PCR compared endogenous AR transcripts in multiple cell types with transgenic AR transcripts in secretory epithelium and provided only a qualitative assessment of transgenic versus wild-type AR expression. Nonetheless, the amount of amplified transgenic AR message was comparable to or greater than that of the wild-type AR.

AR Protein Expression in Transgenic Versus Wild-Type Prostate. The levels of AR protein in prostate tissues were determined by immunoblotting samples from transgenic and wild-type littermate siblings in the initially established 2R1 line. Higher AR levels were detected in transgenic VP versus wild-type VP (Fig. 2*A*, lanes 1 and 2) and in transgenic DLP versus wild-type DLP (Fig. 2*A*, lanes 3 and 4). Interestingly, more AR protein was detected in VP than in DLP, regardless of the source. Because immunoblots were normalized for protein content, this higher level of AR protein may reflect the higher stromal content in DLP (see below). The distribution and level of AR protein in prostates from wild-type littermate controls versus transgenic mice were also assessed by immunohistochemistry in the 2R1 line. As in wild-type mice (Fig. 2 *B* and *C*), the AR in transgenic mice was located in the nuclei of secretory epithelial cells and in scattered stromal cells in VP (Fig. 2*D*) and DLP (Fig. 2*E*). However, the epithelial expression in wild-type glands was

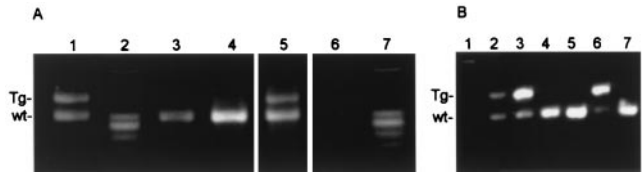

Fig. 1. RT-PCR analysis of Pb-mAR and wild-type tissue RNAs. (*A*) Comparison of endogenous and transgenic AR transcript levels in transgenic founders. Lane 1, 2R1 DLP; lane 2, markers; lane 3, 9R1 DLP; lane 4, 9R1 VP; lane 5, 23R1 VP; lane 6, H_2O negative control; lane 7, markers. (*B*) Comparison of endogenous and transgenic AR transcript levels among tissues in the transgenic lineage 2R1. Lane 1, marker; lane 2, VP; lane 3, DLP; lane 4, liver; lane 5, kidney; lane 6, anterior prostate; lane 7, testis. Positions of the transgenic (Tg) and wild-type (wt) transcripts are indicated. Samples in *A* and *B* were amplified independently under the same conditions. Prostatic and other tissues were dissected and processed separately.

heterogeneous, with many nuclei exhibiting low or undetectable levels of AR immunostaining. In contrast, there was uniform high-level AR expression in transgenic glands. These findings were consistent with the RT-PCR and immunoblotting results and demonstrated that the Pb-mAR transgene augmented AR protein expression in VP and DLP epithelium.

Histological Analysis of mAR Transgenic Mice. Prostates from mice in each of the three founder lines expressing the mAR transgene (2R1, 23R1, and 2R2) were collected for histological study. Mice with dysplastic glands in the VP or DPL were found in all three lineages (see below), whereas dysplasia was not found in any age-matched wild-type littermate controls. This difference in the presence of dysplasia between the transgenic and wild-type mice indicated that the dysplastic lesions were because of the expressed AR and were not the result of insertional mutagenesis. Results from the initially established 2R1 line versus wild-type littermate controls are summarized in Table 1, and examples are shown in Fig. 3. Statistical analysis indicated that the development of dysplasia was significantly greater in the transgenic mice versus wild-type littermate controls (one-tailed Student's *t* test, $P = 0.0023$), as was the development of severe dysplasia (one-tailed Student's *t* test, $P = 0.0062$). The development of dysplasia was strongly age-dependent, as it was not found in mice younger than 12 months.

An example of a moderate to severely dysplastic lesion in the DLP of a 2R1 founder line mouse is illustrated in Fig. 3*A*. Fig. 3*B* is a low-power micrograph of several severely dysplastic lesions in the DLP, some showing a cribriform growth pattern in which intraluminal glands appear to form within the original gland. At higher power the severely dysplastic lesions were further characterized by pronounced epithelial cell crowding, enlarged vesicular nuclei that often contained one or more prominent nucleoli, and apoptotic cells (Fig. 3*C*). These lesions shared the cytological and histological features that characterize high-grade dysplastic lesions in human prostate, generally referred to as high-grade prostatic intraepithelial neoplasia (PIN), which appear to be precursor lesions to PCa (47).

Changes suggestive of the initial stages of microinvasive carcinoma were found arising in a PIN lesion in the DLP of one animal. Because penetration of the basement membrane is a histological hallmark of early invasion, periodic acid/Schiff (PAS) staining was used to assess this feature in high-grade PIN lesions. In Fig. 3*D*, a PAS-stained PIN lesion shows an intact basement membrane that is of uniform thickness in areas with only moderate dysplasia. In contrast, in the center right of this figure there are cells impinging on the basement membrane, possibly in the initial stages of cribriform gland formation, with thinning of the adjacent basement membrane. At the bottom of this figure (higher power in Fig. 3*E*), a cribriform gland is seen protruding into, but not completely penetrating, the basement membrane. This cribriform gland is associated with a marked thinning of the basement membrane. In other PAS-stained PIN lesions, many dysplastic cells with prominent nucleoli appear to be impinging on or embedded in the basement membrane (Fig. 3*F*, arrow). AR immunohistochemistry of replicate sections, as shown in Fig. 3*G*, demonstrated that these cells were strongly AR-positive (arrow). Finally, Fig. 3*H* shows a gland from the 2R2 lineage with hyperplasia and cribriform growth similar to that seen in the 2R1 line.

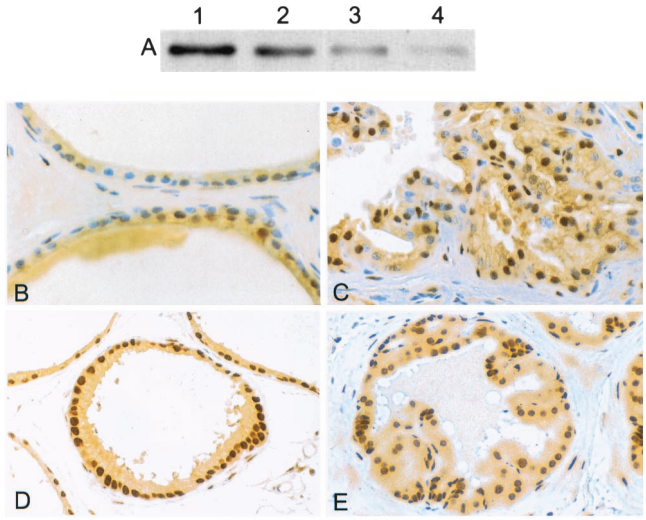

Fig. 2. AR immunoblot and immunohistochemistry in prostate from transgenic and wild-type mice. (*A*) Anti-AR immunoblot. Lanes 1 and 2, VP from transgenic and wild-type, respectively; lanes 3 and 4, DLP from transgenic and wild-type, respectively. (*B–E*) Anti-AR immunohistochemistry. (*B*) Wild-type VP (×500). (*C*) Wild-type DLP (×400). (D) 2R1 transgenic VP (×330). (*E*) 2R1 transgenic DLP (×400).

Increased Proliferation in mAR Transgenic Mice. AR expression in the transgenic mice was relatively uniform by immunohistochemistry, but PIN lesions in these mice were focal and occurred in aged mice. This lack of correspondence between AR expression and PIN lesions indicated that these lesions were likely a result of sporadic secondary genetic or epigenetic events, rather than a primary result of the AR transgene. To identify primary effects of the AR transgene, proliferation of prostate epithelium from Pb-mAR mice was assessed by immunostaining for the Ki-67 proliferation antigen. Ki-67-positive cells (identified by the MIB1 mAb) were rare in prostate epithelium from wild-type mice, with most glands having no positive cells and occasional glands having a single Ki-67-positive cell (not shown). This finding was consistent with a previous report showing an extremely low rate of proliferation (about 0.1%) in normal mouse

Table 1. Histological outcomes of 2R1 lineage Pb-mAR versus wild-type littermate control mice

Lineage	No.	Average age, mo	No dysplasia	Mild/moderate dysplasia	Severe dysplasia
2 R1					
<12 mos	20	5.9	20	0	0
>12 mos	11	16.8	6	1	4
Wild type					
<12 mos	16	5.1	16	0	0
>12 mos	14	14.6	14	0	0

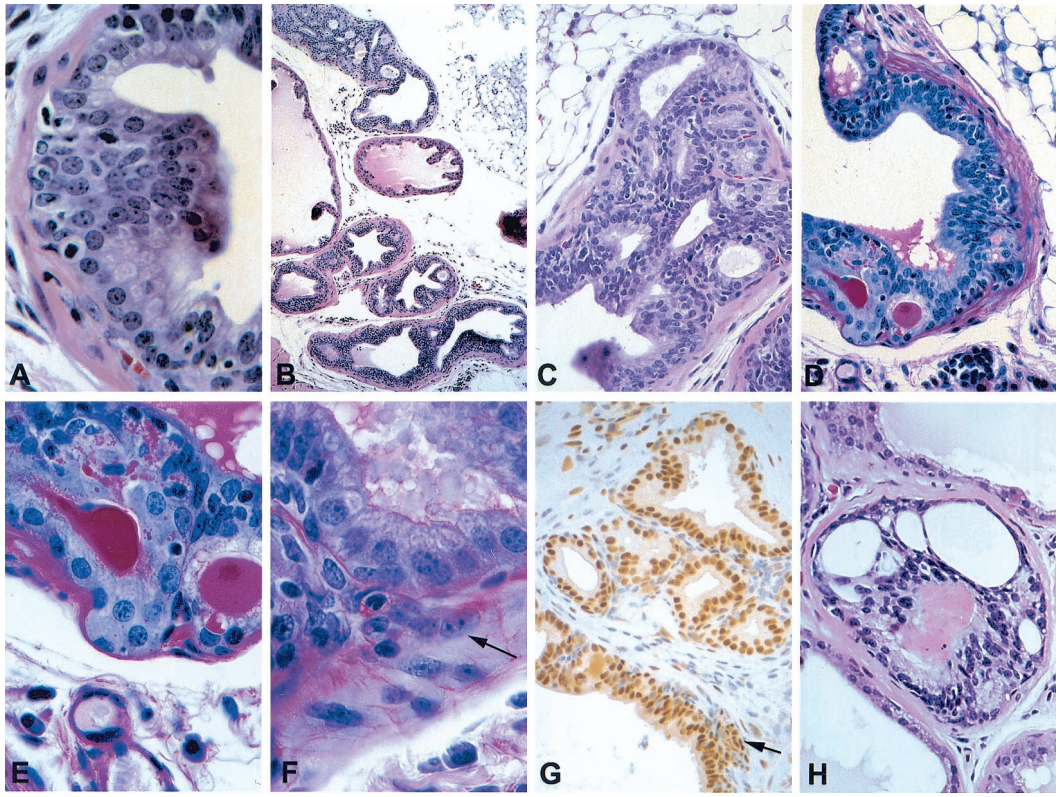

Fig. 3. PIN in prostates from aged Pb-mAR transgenic mice. (*A*) Moderate grade PIN in DLP (hematoxylin/eosin, ×600). (*B*) High-grade PIN in DLP among normal glands (hematoxylin/eosin, ×60). (*C*) High-grade PIN in DLP (hematoxylin/eosin, ×400). (*D*) High-grade PIN in DLP with focal thinning of basement membrane (PAS stain, ×290). (*E*) Higher magnification of sample in *D* (×600). (*F*) High-grade PIN in DLP. The arrow indicates an epithelial nucleus with prominent nucleoli in basement membrane (PAS stain, ×600). (*G*) AR immunohistochemistry of DLP with arrow showing AR-stained epithelial nuclei within or through the basement membrane (×250). (*H*) 2R2 lineage DLP (×330).

prostate epithelium (50). In contrast, glands containing Ki-67-positive cells were frequent in histologically normal VP and DLP from Pb-mAR mice, with one or two positive cells in a gland being common (Fig. 4*A*, arrow), and with some glands having three or more positive cells. Ki-67 staining was particularly high in the ventral prostate from one Pb-mAR mouse (4.5 months old), despite the absence of histologically apparent lesions (Fig. 4*B*). Not surprisingly, dysplastic glands in Pb-mAR mice contained large numbers of Ki-67-positive cells (Fig. 4*C*). Adjacent histologically normal glands also contained large numbers of positive cells, which were possibly because of paracrine factors secreted by the dysplastic gland.

The Ki-67 staining results were quantified in VP and DLP to obtain a proliferative index in prostate glands from wild-type littermate control mice versus histologically normal glands from Pb-mAR transgenic mice that did not have PIN. This quantification was achieved by tabulation of the percentage of total VP or DLP secretory epithelial cells that were Ki-67-positive in a representative group of normal-appearing glands from a series of wild-type and Pb-mAR transgenic mice. Six hundred to 4,000 secretory epithelial cells were typically counted per VP or DLP. Mice with PIN were excluded from this analysis to avoid concerns about paracrine factors from PIN lesions stimulating growth of adjacent glands. Prostates with areas of focal hyperplasia were included in this analysis, but only the histologically normal areas, and not the hyperplastic areas, were counted.

In VP from wild-type littermate control mice, the percentage of Ki-67-positive epithelial cells averaged 0.39% and ranged from 0.36% to 0.44% (Fig. 5, wt VP). In contrast, Pb-mAR transgenic VP averaged 1.76% Ki-67-positive cells and ranged from 0.5% to 8.98%. In DLP, the percentage of Ki-67-positive cells in the wild-type mice averaged 0.21% (Fig. 5, wt DLP). Ki-67-positive cells in DLP from Pb-mAR mice averaged 1.14% and ranged from 0.75% to 2.0%. Therefore, there was a statistically significant increase in epithelial cell proliferation in both the VP and DLP in the Pb-mAR mice relative to wild-type (≈5-fold, one-tailed *t* test: dorsolateral, $P = 0.0007$; ventral, $P = 0.033$). This consistent result in histologically normal-appearing glands indicated that a direct effect of the mAR transgene was to stimulate proliferation of secretory epithelial cells.

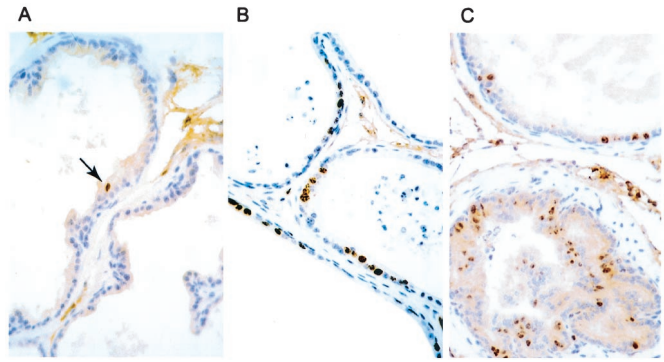

Fig. 4. Higher frequency of expression of the proliferation marker Ki-67 in transgenic mouse prostates. (*A*) Typical view of Ki-67 staining in VP from a transgenic mouse, with a single Ki-67 reactive nucleus (arrow, center) in this specimen with 1.67% Ki-67-positive epithelial cells (×90). (*B*) High Ki-67 staining in histologically normal VP from a 4.5-month-old transgenic mouse (×180). (*C*) High Ki-67 staining in DLP PIN lesion from a transgenic mouse (×100). Wild-type prostate with very rare positive cells is not shown.

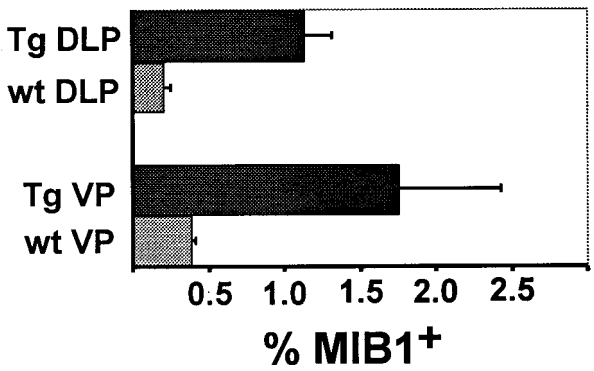

Fig. 5. Comparison of Ki-67 (MIB1)-positive percentages in transgenic versus wild-type prostates.

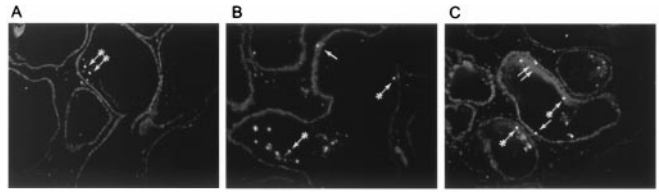

Fig. 6. Higher frequency of apoptosis in VP with higher proliferative index. (*A*) Wild-type VP. (*B*) Transgenic VP with 1.67% Ki-67-positive cells. (*C*) Transgenic VP with 8.98% Ki-67-positive cells. All apoptotic cells located within the prostatic epithelium are indicated by simple arrows, and representative sloughed apoptotic cells are indicated by arrows with asterisks (×125).

Apoptosis in Pb-mAR Prostate Epithelium. Apoptosis was next examined to determine whether the increased proliferation in otherwise normal-appearing prostate epithelium might be balanced by increased apoptosis. Apoptosis was assessed *in situ* by terminal deoxynucleotidyltransferase-mediated UTP end labeling assays and was undetectable in wild-type DLP (not shown) and VP epithelium (Fig. 6*A*). In contrast, apoptotic cells were detected in transgenic DLP (not shown) and VP (Fig. 6*B*, simple arrow, prostate with 1.57% Ki-67-positive cells). More frequent apoptotic cells were seen in a transgenic VP with an extremely high proliferative index (8.98% Ki-67-positive cells) (Fig. 6*C*). There were also sloughed, apoptotic cells in the lumens of glands from all specimens (arrows with asterisks). These were more frequent in the samples with higher proliferation, but whether these primarily represented epithelial cells was not determined. These data indicated that the increased proliferation driven by expression of the mAR transgene was balanced by increased apoptosis in tissue that had normal histology.

Discussion

The majority of prostate cancers are androgen dependent, but androgens have a limited ability to stimulate growth of normal prostate epithelium, and the mechanisms by which AR might contribute to PCa development have not been clear. This study demonstrated that a mAR transgene targeted selectively to prostate secretory epithelium in transgenic mice stimulated proliferation of the epithelium, with the subsequent development of PIN in aged, but otherwise unmanipulated mice. These results provide direct evidence that the AR can function as a positive regulator of proliferation in normal prostate secretory epithelium and stimulate the development of neoplasia. In conjunction with epidemiological data linking increased AR activity and PCa risk, these findings indicate a direct role for the AR in promoting PCa development in humans.

The earliest alteration observed in Pb-mAR mouse prostates was a substantial 5-fold increase in the proliferation of secretory epithelial cells, as evidenced by Ki-67 immunostaining, in the absence of histological abnormalities. This pattern was seen throughout the VP and DLP in all Pb-mAR mice examined and occurred in young and old mice, indicating that it was a direct effect of the transgene. Proliferation in these glands was associated with increased apoptosis, accounting for the absence of hyperplasia. In contrast, PIN lesions were focal and increased with age, indicating that they reflected the occurrence of additional secondary genetic or epigenetic events. Taken together, these findings suggest that the PCa susceptibility associated with increased AR expression is because of a balanced increase in proliferation and apoptosis, with a resultant increase in the frequency of secondary transforming events. It is not yet clear whether these secondary events involve changes in proliferation, apoptosis, or both, but studies in human PCa have found that increased rates of both proliferation and apoptosis correlate with increased malignant potential (51, 52).

The AR has been shown to regulate or interact with a number of proteins that control cell growth. The growth-inhibitory effects of the AR may reflect its ability to stimulate the p21 cyclin-dependent kinase promoter (53) or bind cyclins D or E (54–56). In contrast, the reported interaction between AR and retinoblastoma protein (Rb) could stimulate growth by decreasing the inhibition of E2F (57, 58). The AR may also interact with or regulate multiple other proteins that can affect cell growth (59–64). Therefore, as indicated by the Pb-mAR mice, the level of AR expression and/or activity may be an important determinant of its effects on cell growth. The relative increase in AR activity in the Pb-mAR mice is likely greater than what occurs in men at high risk versus low risk for PCa, accounting for the large effects on proliferation and development of PIN in these mice. Nonetheless, the results indicate that modest prolonged increases in AR activity because of androgen levels or AR polymorphisms can account for an increased lifetime risk for PCa. Moreover, the results provide support for PCa prevention therapies such as finasteride that are designed to decrease AR activity in prostate epithelium.

AR expression is not increased in human high-grade PIN or primary PCa (25, 65–67). This finding is consistent with the interpretation that the increased AR expression in the Pb-mAR mice is not by itself a transforming event, and that PIN in these mice is the result of additional steps. Such a multistep process is certainly consistent with our current understanding of how PCa develops in humans. Analyses of human prostate cancers have suggested multiple possible secondary genetic or epigenetic events that could further increase cell proliferation and/or increase cell survival (68). It will clearly be of interest to identify such additional changes in this Pb-mAR model.

There are now multiple murine transgenic and knockout models that develop varying degrees of prostate hyperplasia, dysplasia, or cancer (69, 70). In some cases these models reflect events that appear to contribute to human PCa, such as stimulation of the insulin-like growth factor-1 axis (71) or loss of PTEN and p27 (50, 72), although PTEN and p27 loss may be late events in human PCa development. Other models introduce potent oncogenes, in particular, the simian virus 40 TAg (46), but do not reflect mechanisms that occur commonly in PCa as primary events. We propose that like the adult human male, Pb-mAR transgenic mice represent a model that is genetically susceptible to androgen-dependent PCa, but which has not acquired secondary transforming events. Therefore, this may be a biologically relevant model in which to identify AR functions that contribute to PCa and to identify secondary transforming genetic and epigenetic events. Furthermore, this model may prove useful for the testing of drugs, diet, or other therapies designed for the prevention of PCa.

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1. Catalona, W. J. (1994) *N. Engl. J. Med.* **331,** 996–1004.
2. Bubley, G. J. & Balk, S. P. (1996) *Hematol. Oncol. Clin. North Am.* **10,** 713–725.
3. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M. & Chambon, P. (1995) *Cell* **83,** 835–839.
4. Brinkmann, A. O., Blok, L. J., de Ruiter, P. E., Doesburg, P., Steketee, K., Berrevoets, C. A. & Trapman, J. (1999) *J. Steroid Biochem. Mol. Biol.* **69,** 307–313.
5. Quigley, C. A., De Bellis, A., Marschke, K. B., el Awady, M. K., Wilson, E. M. & French, F. S. (1995) *Endocr. Rev.* **16,** 271–321.
6. Huggins, C. & Hodges, C. V. (1972) *CA Cancer J. Clin.* **22,** 232–240.
7. Jenster, G. (1999) *Semin. Oncol.* **26,** 407–421.
8. Culig, Z., Hobisch, A., Cronauer, M. V., Cato, A. C., Hittmair, A., Radmayr, C., Eberle, J., Bartsch, G. & Klocker, H. (1993) *Mol. Endocrinol.* **7,** 1541–1550.
9. Suzuki, H., Sato, N., Watabe, Y., Masai, M., Seino, S. & Shimazaki, J. (1993) *J. Steroid Biochem. Mol. Biol.* **46,** 759–765.
10. Taplin, M. E., Bubley, G. J., Shuster, T. D., Frantz, M. E., Spooner, A. E., Ogata, G. K., Keer, H. N. & Balk, S. P. (1995) *N. Engl. J. Med.* **332,** 1393–1398.
11. Visakorpi, T., Hyytinen, E., Koivisto, P., Tanner, M., Keinanen, R., Palmberg, C., Palotie, A., Tammela, T., Isola, J. & Kallioniemi, O. P. (1995) *Nat. Genet.* **9,** 401–406.
12. Taplin, M. E., Bubley, G. J., Ko, Y. J., Small, E. J., Upton, M., Rajeshkumar, B. & Balk, S. P. (1999) *Cancer Res.* **59,** 2511–2515.
13. Gann, P. H., Hennekens, C. H., Ma, J., Longcope, C. & Stampfer, M. J. (1996) *J. Natl. Cancer Inst.* **88,** 1118–1126.
14. Shaneyfelt, T., Husein, R., Bubley, G. & Mantzoros, C. S. (2000) *J. Clin. Oncol.* **18,** 847–853.
15. Hardy, D. O., Scher, H. I., Bogenreider, T., Sabbatini, P., Zhang, Z. F., Nanus, D. M. & Catterall, J. F. (1996) *J. Clin. Endocrinol. Metab.* **81,** 4400–4405.
16. Giovannucci, E., Stampfer, M. J., Krithivas, K., Brown, M., Dahl, D., Brufsky, A., Talcott, J., Hennekens, C. H. & Kantoff, P. W. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 3320–3323.
17. Stanford, J. L., Just, J. J., Gibbs, M., Wicklund, K. G., Neal, C. L., Blumenstein, B. A. & Ostrander, E. A. (1997) *Cancer Res.* **57,** 1194–1198.
18. Hakimi, J. M., Schoenberg, M. P., Rondinelli, R. H., Piantadosi, S. & Barrack, E. R. (1997) *Clin. Cancer Res.* **3,** 1599–1608.
19. Nam, R. K., Elhaji, Y., Krahn, M. D., Hakimi, J., Ho, M., Chu, W., Sweet, J., Trachtenberg, J., Jewett, M. A. & Narod, S. A. (2000) *J. Urol.* **164,** 567–572.
20. Hsing, A. W., Gao, Y. T., Wu, G., Wang, X., Deng, J., Chen, Y. L., Sesterhenn, I. A., Mostofi, F. K., Benichou, J. & Chang, C. (2000) *Cancer Res.* **60,** 5111–5116.
21. Chamberlain, N. L., Driver, E. D. & Miesfeld, R. L. (1994) *Nucleic Acids Res.* **22,** 3181–3186.
22. Choong, C. S., Kemppainen, J. A., Zhou, Z. X. & Wilson, E. M. (1996) *Mol. Endocrinol.* **10,** 1527–1535.
23. Irvine, R. A., Ma, H., Yu, M. C., Ross, R. K., Stallcup, M. R. & Coetzee, G. A. (2000) *Hum. Mol. Genet.* **9,** 267–274.
24. Cussenot, O., Berthon, P., Cochand-Priollet, B., Maitland, N. J. & Le Duc, A. (1994) *Exp. Cell Res.* **214,** 83–92.
25. Leav, I., McNeal, J. E., Kwan, P. W., Komminoth, P. & Merk, F. B. (1996) *Prostate* **29,** 137–145.
26. Peehl, D. M. & Stamey, T. A. (1986) *In Vitro Cell Dev. Biol.* **22,** 82–90.
27. Grant, E. S., Batchelor, K. W. & Habib, F. K. (1996) *Prostate* **29,** 339–349.
28. Berthon, P., Waller, A. S., Villette, J. M., Loridon, L., Cussenot, O. & Maitland, N. J. (1997) *Int. J. Cancer* **73,** 910–916.
29. Marcelli, M., Haidacher, S. J., Plymate, S. R. & Birnbaum, R. S. (1995) *Endocrinology* **136,** 1040–1048.
30. Heisler, L. E., Evangelou, A., Lew, A. M., Trachtenberg, J., Elsholtz, H. P. & Brown, T. J. (1997) *Mol. Cell. Endocrinol.* **126,** 59–73.
31. Shen, R., Sumitomo, M., Dai, J., Harris, A., Kaminetzky, D., Gao, M., Burnstein, K. L. & Nanus, D. M. (2000) *Endocrinology* **141,** 1699–1704.
32. Wu, H. C., Hsieh, J. T., Gleave, M. E., Brown, N. M., Pathak, S. & Chung, L. W. (1994) *Int. J. Cancer* **57,** 406–412.
33. Kokontis, J. M., Hay, N. & Liao, S. (1998) *Mol. Endocrinol.* **12,** 941–953.
34. Culig, Z., Hoffmann, J., Erdel, M., Eder, I. E., Hobisch, A., Hittmair, A., Bartsch, G., Utermann, G., Schneider, M. R., Parczyk, K., *et al.* (1999) *Br. J. Cancer* **81,** 242–251.
35. Cunha, G. R. (1984) *Prog. Clin. Biol. Res.* **145,** 81–102.
36. Yan, G., Fukabori, Y., Nikolaropoulos, S., Wang, F. & McKeehan, W. L. (1992) *Mol. Endocrinol.* **6,** 2123–2128.
37. Peehl, D. M. & Rubin, J. S. (1995) *World J. Urol.* **13,** 312–317.
38. Lucia, M. S., Bostwick, D. G., Bosland, M., Cockett, A. T., Knapp, D. W., Leav, I., Pollard, M., Rinker-Schaeffer, C., Shirai, T. & Watkins, B. A. (1998) *Prostate* **36,** 49–55.
39. McCormick, D. L., Rao, K. V., Dooley, L., Steele, V. E., Lubet, R. A., Kelloff, G. J. & Bosland, M. C. (1998) *Cancer Res.* **58,** 3282–3288.
40. Noble, R. L. (1982) *Int. Rev. Exp. Pathol.* **23,** 113–159.
41. Leav, I., Merk, F. B., Kwan, P. W. & Ho, S. M. (1989) *Prostate* **15,** 23–40.
42. Bruchovsky, N., Lesser, B., Van Doorn, E. & Craven, S. (1975) *Vitam. Horm.* **33,** 61–102.
43. Quarmby, V. E., Yarbrough, W. G., Lubahn, D. B., French, F. S. & Wilson, E. M. (1990) *Mol. Endocrinol.* **4,** 22–28.
44. Dai, J. L. & Burnstein, K. L. (1996) *Mol. Endocrinol.* **10,** 1582–1594.
45. Greenberg, N. M., DeMayo, F. J., Sheppard, P. C., Barrios, R., Lebovitz, R., Finegold, M., Angelopoulou, R., Dodd, J. G., Duckworth, M. L., Rosen, J. M., *et al.* (1994) *Mol. Endocrinol.* **8,** 230–239.
46. Greenberg, N. M., DeMayo, F., Finegold, M. J., Medina, D., Tilley, W. D., Aspinall, J. O., Cunha, G. R., Donjacour, A. A., Matusik, R. J. & Rosen, J. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3439–3443.
47. Bostwick, D. G., Pacelli, A. & Lopez-Beltran, A. (1996) *Prostate* **29,** 117–134.
48. Faber, P. W., King, A., van Rooij, H. C., Brinkmann, A. O., de Both, N. J. & Trapman, J. (1991) *Biochem. J.* **278,** Part 1, 269–278.
49. Rennie, P. S., Bruchovsky, N., Leco, K. J., Sheppard, P. C., McQueen, S. A., Cheng, H., Snoek, R., Hamel, A., Bock, M. E., MacDonald, B. S., *et al.* (1993) *Mol. Endocrinol.* **7,** 23–36.
50. Di Cristofano, A., Pesce, B., Cordon-Cardo, C. & Pandolfi, P. P. (1998) *Nat. Genet.* **19,** 348–355.
51. Wheeler, T. M., Rogers, E., Aihara, M., Scardino, P. T. & Thompson, T. C. (1994) *J. Cell Biochem. Suppl.* **19,** 202–207.
52. Aihara, M., Scardino, P. T., Truong, L. D., Wheeler, T. M., Goad, J. R., Yang, G. & Thompson, T. C. (1995) *Cancer (Philadelphia)* **75,** 522–529.
53. Lu, S., Liu, M., Epner, D. E., Tsai, S. Y. & Tsai, M. J. (1999) *Mol. Endocrinol.* **13,** 376–384.
54. Knudsen, K. E., Cavenee, W. K. & Arden, K. C. (1999) *Cancer Res.* **59,** 2297–2301.
55. Yamamoto, A., Hashimoto, Y., Kohri, K., Ogata, E., Kato, S., Ikeda, K. & Nakanishi, M. (2000) *J. Cell Biol.* **150,** 873–880.
56. Reutens, A. T., Fu, M., Wang, C., Albanese, C., McPhaul, M. J., Sun, Z., Balk, S. P., Janne, O. A., Palvimo, J. J. & Pestell, R. G. (2001) *Mol. Endocrinol.* **15,** 797–811.
57. Lu, J. & Danielsen, M. (1998) *J. Biol. Chem.* **273,** 31528–31533.
58. Yeh, S., Miyamoto, H., Nishimura, K., Kang, H., Ludlow, J., Hsiao, P., Wang, C., Su, C. & Chang, C. (1998) *Biochem. Biophys. Res. Commun.* **248,** 361–367.
59. Lu, S., Tsai, S. Y. & Tsai, M. J. (1997) *Cancer Res.* **57,** 4511–4516.
60. Chi, S. G., deVere White, R. W., Muenzer, J. T. & Gumerlock, P. H. (1997) *Clin. Cancer Res.* **3,** 1889–1897.
61. Gregory, C. W., Hamil, K. G., Kim, D., Hall, S. H., Pretlow, T. G., Mohler, J. L. & French, F. S. (1998) *Cancer Res.* **58,** 5718–5724.
62. Ye, D., Mendelsohn, J. & Fan, Z. (1999) *Clin. Cancer Res.* **5,** 2171–2177.
63. Lee, D. K., Duan, H. O. & Chang, C. (2000) *J. Biol. Chem.* **275,** 9308–9313.
64. Truica, C. I., Byers, S. & Gelmann, E. P. (2000) *Cancer Res.* **60,** 4709–4713.
65. Tsuji, M., Kanda, K., Murakami, Y., Kurokawa, Y., Kanayama, H., Sano, T. & Kagawa, S. (1999) *J. Med. Invest.* **46,** 35–41.
66. Harper, M. E., Glynne-Jones, E., Goddard, L., Mathews, P. & Nicholson, R. I. (1998) *J. Pathol.* **186,** 169–177.
67. Sweat, S. D., Pacelli, A., Bergstralh, E. J., Slezak, J. M. & Bostwick, D. G. (1999) *J. Urol.* **161,** 1229–1232.
68. Ozen, M. & Pathak, S. (2000) *Anticancer Res.* **20,** 1905–1912.
69. Green, J. E., Greenberg, N. M., Ashendel, C. L., Barrett, J. C., Boone, C., Getzenberg, R. H., Henkin, J., Matusik, R., Janus, T. J. & Scher, H. I. (1998) *Prostate* **36,** 59–63.
70. Sharma, P. & Schreiber-Agus, N. (1999) *Oncogene* **18,** 5349–5355.
71. DiGiovanni, J., Kiguchi, K., Frijhoff, A., Wilker, E., Bol, D. K., Beltran, L., Moats, S., Ramirez, A., Jorcano, J. & Conti, C. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 3455–3460.
72. Di Cristofano, A., De Acetis, M., Koff, A., Cordon-Cardo, C. & Pandolfi, P. P. (2001) *Nat. Genet.* **27,** 222–224.