Growth, regeneration, and tumorigenesis of the prostate activates the PSCA promoter

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Contributed by Owen N. Witte, October 29, 2001

The prostate gland undergoes dramatic changes in growth status during normal physiologic development, following androgen administration to castrate animals, and during tumor development. The prostate stem cell antigen (PSCA, named for its strong sequence homology to the thymocyte marker stem cell antigen 2) is a cell surface molecule associated with human and murine prostate cancer. To help define the regulation of this molecule, we created a transgenic mouse strain, which uses the human PSCA promoter region to control the expression of enhanced green fluorescent protein (GFP). Expression of GFP was detected in mid-gestation following the appearance of prostatic buds from the urogenital sinus. In adult mice, GFP expression was restricted to a subset of cells located in the distal tips of the glands. GFP expression increased during puberty and regeneration driven by androgen and associated with expansive growth of the prostate. GFP-positive cells coexpressed markers associated with both basal and secretory cells in the human prostate. Prostate carcinogenesis driven by T antigen in the *t*ransgenic *a*denocarcinoma of the *m*ouse *p*rostate (TRAMP) model results in an increased percentage and intensity level for PSCA promoter-driven GFP-positive cells. This transgenic system helps define the range of cellular changes associated with altered expression of PSCA, shows that transcriptional control is a major component regulating PSCA levels, and provides a useful tool to study subpopulations of prostate epithelial cells and factors that regulate the PSCA promoter.

Development and Heterogeneity of the Mouse Prostate

The adult mouse prostate consists of paired anterior (also known as the coagulating gland) dorsolateral (DLP) and ventral (VP) lobes that arise as epithelial buds off the urogenital sinus around embryonic day 17. These prostatic buds elongate and branch rapidly during the first 3 weeks of postnatal life. Significant growth and maturation occurs during puberty, at age 5–6 weeks (1, 2). The prostate is an androgen-dependent organ that is capable of self-renewal. Castration results in prostatic involution, whereas re-administration of androgen leads to complete regeneration of the normal epithelium from a putative population of stem cells (3, 4). The identity and location of such cells remains speculative, in part because the cellular and regional organization of the prostate is poorly understood.

The rodent (and human) prostate epithelium is comprised principally of luminally located secretory and subjacent basal cells. One hypothesis is that basal cells are the precursors of terminally differentiated secretory cells (5). This hypothesis is based on the differential loss of secretory cells following castration and the ability of basal cells to give rise to luminal cells *in vitro* and *in vivo* (6, 7). A relationship between basal and secretory cells has also been suggested by the identification of cells that are immunophenotypically intermediate between basal and secretory cells upon androgen-induced prostate regeneration (8). Other investigators, however, have argued that basal and secretory cells may not be connected in a direct precursor–progeny manner, because both basal and secretory cells are present after castration and both can proliferate in response to androgen replacement (9, 10).

The prostatic ducts are organized along a proximal to distal axis from the urethra out to the distal tips. A majority of proliferating cells are located at the tips of the ducts, whereas the proximal ducts attached to the urethra contain a high percentage of apoptotic cells (11, 12). Secretory activity has been proposed to be concentrated in the intermediate regions of the duct. The distal tips of the dorsolateral prostates of rats could undergo massive epithelial growth when recombined with urogenital sinus mesenchyme, suggesting the presence of stem cells within the ductal tips (13). In contrast, telomerase activity is significantly higher in the proximal ducts than in the distal tips, suggesting the existence of stem cells in the proximal regions of the ductal structure (14). Finally, a number of authors have reported regional variations in gene expression. CD44, for example, a marker of human prostate basal cells, localizes to a subset of luminal cells in the ductal tips of the mouse prostate (15).

The relationship of the different prostate cell types and ductal regions to prostate carcinogenesis is not known. A number of investigators have generated transgenic models of prostate cancer by selectively targeting SV40 T antigen to the prostate by using the androgen-dependent C3 and probasin promoters (16–18). A hallmark of these models is that they are androgen dependent, because cancers do not occur in mice castrated at a young age. Because C3 and probasin are secretory cell differentiation antigens, it is presumed that these cancers originate in secretory cells. However, the exact identity and location of the transformed cell type in these models is not known. Recently, DiGiovanni *et al.* (19) developed a transgenic model of prostate cancer by targeting insulin growth factor receptor 1 to prostatic basal cells by using the cytokeratin 5 promoter. These existing models suggest that prostate cancer could arise from multiple epithelial populations (basal and secretory) within the prostate, but do not rule out the possibility that cancers could have arisen from an intermediate population in which the CK5, probasin, and C3 promoters could all be active.

Prostate Stem Cell Antigen in the Normal and Malignant Prostate

Our understanding of the complexity of prostatic cellular and regional heterogeneity has been hindered by a lack of markers associated with normal and malignant prostate growth. Prostate stem cell antigen (PSCA) is a member of the Ly-6/Thy-1 family of GPI-anchored glycoproteins (20). It was named because of its

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Abbreviations: GFP, green fluorescent protein; dpc, days before conception; TRAMP, transgenic adenocarcinoma of the mouse prostate; PSCA, prostate stem cell antigen; mPSCA, murine PSCA.

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close homology to stem cell antigen 2 (sca-2), a marker of immature thymic lymphocytes. In humans, PSCA expression is largely restricted to the prostate, bladder, and stomach. In the prostate, PSCA mRNA is most readily detected in a subset of basal cells, whereas PSCA protein is detected in both basal and secretory cells. PSCA is expressed in almost all cases of high-grade prostate intraepithelial neoplasia (HGPIN) and invasive prostate cancer and is overexpressed in ≈40% of localized and 100% of bone metastatic (nine of nine) prostate cancers examined (21). PSCA is a candidate target for the immunotherapy of prostate cancer. Monoclonal antibodies directed against PSCA have been shown to inhibit prostate cancer growth and metastasis in human xenograft models (22).

A murine homologue of PSCA has been identified that is expressed in the adult prostate and bladder (20). Murine PSCA (mPSCA) is expressed on ≈20% of normal young adult prostate epithelial cells by flow cytometry and the percentage of positive cells decreases with age (23). Similar results have been reported for PSCA mRNA expression by using *in situ* hybridization (24). The percentage of cells expressing mPSCA during carcinogenesis increases dramatically. In the *t*ransgenic *a*denocarcinoma of the *m*ouse *p*rostate model (TRAMP, generated by expression of large T antigen in the prostate by the probasin promoter), for example, 60% or more of cells stain strongly for PSCA compared with <10% in matched normal prostates of the same age (16, 23, 24). Importantly, PSCA expression is also detected in mouse HGPIN, a putative precursor of invasive cancer. This expansion of PSCA-positive cells in both human and murine prostate cancer suggests that PSCA may mark a subset of cells associated with prostate transformation. In addition, these observations suggest that study of mPSCA expression in normal and malignant mouse prostate may provide insights into PSCA gene regulation in human prostate cancer.

Reasoning that the PSCA promoter might provide a means to define and target a subpopulation of cells associated with human and mouse prostate carcinogenesis *in vivo*, a transgenic mouse model was generated in which the human PSCA promoter was used to drive expression of enhanced green fluorescent protein (GFP) in the prostate. GFP expression in these mice was restricted to a subset of epithelial cells within the distal tips of the mature prostatic duct, consistent with the limited expression of PSCA in the normal prostate. GFP expression increased and expanded during periods of active ductal growth, such as puberty and after administration of testosterone to castrated mice, but was barely detectable after castration-induced prostate regression. There was also an increase in the percentage and intensity of GFP-positive cells in prostate cancer. To characterize these cells, GFP-positive cells were isolated by flow cytometry. Analysis of gene expression in these cells revealed that they coexpress markers associated with both basal and secretory cells in the human prostate, suggesting that these cells are of intermediate differentiation. These results demonstrate that the PSCA promoter can effectively drive gene expression to a growth-responsive prostate epithelial cell population that is associated with prostate tumorigenesis in the TRAMP mouse. These results also suggest that PSCA up-regulation in cancer is a transcriptional event regulated at the level of the PSCA promoter.

Materials and Methods

Construction of Expression Plasmids. A human PSCA genomic clone has been described (20). This 14-kb PSCA genomic DNA fragment was used to generate a fragment containing the 9 kb of 5′ flanking sequence and 5 bp of untranslated region by high-fidelity PCR (Roche Diagnostics) using the *Hin*dII/3′ and T7 primers. The 9-kb sequence was cloned into the *Sma*I and *Hin*dIII sites of the promoterless luciferase vector pGL3-basic (Promega). The 3-kb and 1-kb deletion constructs were obtained by digestion of the pGL3-PSCA (9 kb) plasmid with *Bam*HI and *Kpn*I, respectively, followed by self ligation.

The PSCA (9 kb)-GFP transgenic vector was constructed by inserting the 9-kb upstream sequence (obtained by PCR using the *Eco*RI/3′ and T7 primers) into the *Eco*RI site of a promoterless enhanced GFP vector (CLONTECH). Sequences of the PCR primers are listed below. *Hin*dIII/3′ primer, 5′-GGGAAGCTTG-CACAGCCTTCAGGGTC-3′; *Eco*RI/3′ primer, 5′-GGGAAT-TCGCACAGCCTTCAGGTC-3′.

Cell Cultures, DNA Transfection, and Luciferase Assay. LNCaP cells were originally obtained from the American Type Culture Collection and primary normal prostate epithelial cells (PrEC) were purchased from Clonetics (BioWhittaker, San Diego). LNCaP cells were maintained in RPMI medium 1640 supplemented with 5% FCS, whereas PrEC were maintained in PREGM growth medium (Clonetics). For examination of androgen induction, the synthetic androgen R1881 (DuPont/NEN) was added to a final concentration of 10 nM. Cells were transfected by using FuGene6 (Boehringer Mannheim) according to the lipofection method described by the manufacturer. Transfections were performed in triplicate and repeated at least three times. Cells were collected 48 h after transfection and firefly and *Renilla* luciferase activities measured by luminometry using 10 μl of cell lysate.

Generation of Transgenic Mice and Animal Handling. Transgenic mouse lines were produced by injecting the purified *Sac*I and *Afl*II fragment into mouse fertilized eggs derived from intercrosses of (C57BL/6XC3H) hybrid mice. Founders were identified by Southern blot analysis of tail DNAs, using enhanced GFP cDNA as a probe. Founders were bred to C57BL/6 mice to generate offspring that were subjected to GFP analysis. Mouse studies were performed according to the guidelines set forth by the University of California, Los Angeles, Animal Research Committee. Prostates were harvested as described (17). Developmental studies were initiated by mating a male hemizygous transgenic mouse to a female C57BL/6 mouse. The day the vaginal plug was first noted was scored as day 0 of gestation. Mice were surgically castrated by using standard surgical technique. For hormone restoration analysis, a testosterone pellet (12.5 mg/pellet) was administered s.c. and then every 2 weeks as indicated.

Histology and Immunohistochemistry. Tissues from transgenic and nontransgenic mice were dissected and mounted in plates supplied with PBS. An Olympus (New Hyde Park, NY) microscope equipped for epifluorescence with a BP490-FY 455 filter set was used to visualize GFP. For immunohistochemistry, tissues were fixed in 10% formalin for 12 h, followed by paraffinization. Sections were processed for immunohistochemistry by using the StreptAB-Complex/HRP kit (Dako) according to the manufacturer's guidelines. Polyclonal antibody against GFP (Molecular Probes) was used at a concentration of 10 ng/ml. Sections were counterstained in hematoxylin and mounted in Permount.

Gene Expression Analysis. Prostate tissues were minced and digested with collagenase I (Sigma) at a concentration of 1 mg/ml for 1 h at room temperature. Large debris were excluded with a 0.45-μm cell strainer. Single cell suspensions were analyzed and sorted into subpopulations with FACS Vantage (Becton Dickinson). Reverse transcriptase (RT)-PCR and cDNA amplification was used to detect the expression of marker genes in the sorted cell populations. Briefly, polyA+ RNAs were obtained from 1×10^5 sorted cells by using the QuickPrep Micro mRNA Purification kit (Amersham Pharmacia). All of the RNAs were converted to cDNA by using the SMART cDNA construction kit (CLONTECH), followed by PCR-based amplification of cDNA. Sequences present in the cDNA were detected by PCR with gene-specific primer pairs and agarose electrophoresis of the reaction products. PCR cycle number for each marker gene was used in the linear range. β-Actin was used as a control. DNA sequences of the primer pairs are as follows:

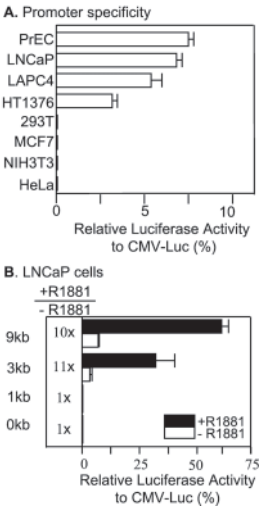

Fig. 1. Characterization of the human PSCA promoter. (*A*) *In vitro* tissue specificity of the human PSCA promoter. Various cell types were transiently transfected with a luciferase reporter construct carrying the 9-kb flanking sequence of the PSCA gene as described in *Materials and Methods*. Relative luciferase activity is reported as a percent of the luciferase activity driven by the CMV promoter for each cell type. Standard error of the relative activity is represented by the horizontal stripe. (*B*) Identification of cis elements within the 9-kb PSCA promoter sequence that confer the androgen responsiveness in LNCaP cells. LNCaP cells were transfected with various PSCA–luciferase constructs in the absence or presence of synthetic androgen, R1881. Activity in the presence or absence of R1881 was represented by an open or solid bar, respectively. Hormone induction is given at the left of the bars.

β-actin-5′, GACTACCTCATGAAGATCCT; β-actin-3′, GCGGATGTCCACGTCACACT; GFP-5′, CTGGTCGAGCTGGACGGCGACG; GFP-3′, CACGAACTCCAGCAGGACCATG; mPSCA-5′, TTCTCCTGCTGGCCACCTAC; mPSCA-3′, GCAGCTCATCCCTTCACAAT; Estrogen receptor β-5′, GTCCTGCTGTGATGAACTACAGAGTG; Estrogen receptor β-3′, CTCTTGGCGCTTGGACTAGTAAC; CD44–5′, ATCACCGACAGCACAGACAGAATCCCT; CD44–3′, ATCTGATTCAGATCCATGAGTGGTATG; PROBASIN-5′, ATCATCCTTCTGCTCACACTGCATG; PROBASIN-3′, ACAGTTGTCCGTGTCCATGATACGC; ETS2–5′, TCGGCTCAACACCGTCAATGTCAA; ETS2–3′, TTCTGTATCAGGCTGGACGCCCAG.

Results

Cloning and Characterization of the Human PSCA Promoter Region. A 14-kb genomic fragment containing 9 kb of sequence upstream from the human PSCA transcription start site was isolated and characterized for promoter activity. Tissue specificity was examined by cloning the 9-kb fragment upstream of a luciferase reporter gene and testing its activity in a variety of cell lines (Fig. 1*A*). Consistent with the known expression of human PSCA in prostate and bladder, the 9-kb fragment could drive luciferase expression in prostate (PrEC, LNCaP, and LAPC 4) and bladder (HT1376) cell lines, but not in kidney (293T), breast (MCF7), fibroblast (NIH 3T3), or uterine (HeLa) cell lines (Fig. 1*A*). These results suggest that the 9-kb region contains regulatory elements that are active in cell lines derived from PSCA-positive tissues.

The 9-kb PSCA promoter region was further characterized by using deletion constructs. Two prostatic cell lines, PrEC and LNCaP, were used for these experiments. PrEC cells are primary cultures of normal prostate epithelium, which do not express androgen receptor (AR) or PSA (25). LNCaP is an androgen-responsive prostate cancer cell line that expresses both AR and PSA. Constructs containing 1, 3, or 9 kb of upstream sequence were transiently transfected into these cells and assayed for luciferase activity. The 9-kb fragment drove luciferase expression both in PrEC and LNCaP, whereas the 3-kb fragment was active only in LNCaP, suggesting that sequences between 3 and 9 kb were sufficient to drive expression even in the absence of androgen (data not shown). The androgen responsiveness of these constructs was tested by comparing activity in the presence and absence of exogenous androgen. As shown in Fig. 1*B*, the 3- and 9-kb segments responded positively to androgen, whereas the 1-kb construct showed no responsiveness. These results show that the PSCA promoter region is both androgen independent and androgen responsive.

The 9-kb PSCA Promoter/Enhancer Region Directs Reporter Gene Expression to the Prostate of Transgenic Mice. Transgenic mice expressing enhanced GFP under the control of the 9-kb PSCA promoter construct were generated to test whether the PSCA promoter could target reporter gene expression to the murine prostate. GFP was selected because of its ease of detection by immunohistochemistry, fluorescent microscopy, and flow cytometry. Transgenic constructs were injected into the pronuclei of C57BL/6 $\times$ C3H hybrid mice. Seven pups contained the transgene by Southern blot and PCR analysis of tail DNA and were bred. Eight-week-old transgenic and nontransgenic offspring were analyzed for GFP expression by RT-PCR and fluorescent microscopy. Prostatic GFP expression was detected in three lines, and the founder with the highest level of GFP expression was maintained.

Among the positive founder lines, all had detectable GFP expression in the most superficial layer of the epidermis, which extinguished with age (data not shown). This expression in skin is reminiscent of the endogenous mPSCA gene, which was reported to be expressed transiently in fetal skin from days E15–E17 (24). One founder also had GFP expression in the transitional epithelium of the bladder. No other tissue had detectable GFP protein or RNA expression. Lung and liver had no detectable protein or RNA expression (data not shown). Stomach, brain, kidney, spleen, bone marrow, seminal vesicle, penis, testis, pancreas, and intestine had no detectable GFP protein expression (RNAs for these tissues were not examined; data not shown). No extraprostatic expression was seen in the four negative founders. These results demonstrate that the 9-kb PSCA promoter can drive reporter gene expression to a restricted number of tissues *in vivo*, consistent with its expression in normal adults.

GFP Expression During Prostate Ductal Morphogenesis, Puberty, and Aging. To examine the kinetics of PSCA-driven GFP expression during normal development, we first examined 16–18 dpc (days post conception) embryos, at which point the prostate epithelium buds off of the urogenital sinus into the surrounding mesenchyme (Fig. 2*A*). Although GFP expression could not be detected by direct fluorescent microscopy at this time point, it could be seen immunohistochemically at 18 dpc in the epithelium of the urogenital sinus by using anti-GFP antibody (Fig. 2*B*). No GFP expression was detected in the solid mass of undifferentiated prostatic bud cells first visualized at day 18 (Fig. 2*B*). However, GFP expression could be seen in the proximal canalizing region of the prostatic buds, where the epithelium first begins to form ductal and glandular structure.

There is extensive branching morphogenesis of the mouse prostate in the first 3 weeks after birth (2). At one week of age, low level GFP expression was detected in all but the most distal regions of the prostatic duct, where canalization had not yet occurred (Fig. 2*C*). GFP expression increased dramatically during puberty (i.e., 5 weeks of age) but, in contrast to 1 week old mice, was now detected in the distal tips but not in the proximal regions of the duct (Fig. 2*D*). Following puberty, GFP expression became increasingly restricted to columnar cells in the distal regions of the prostatic ducts and this pattern became more accentuated as mice aged (Fig. 2 *E* and *F*). These results demonstrate that PSCA-driven GFP marks a sub-

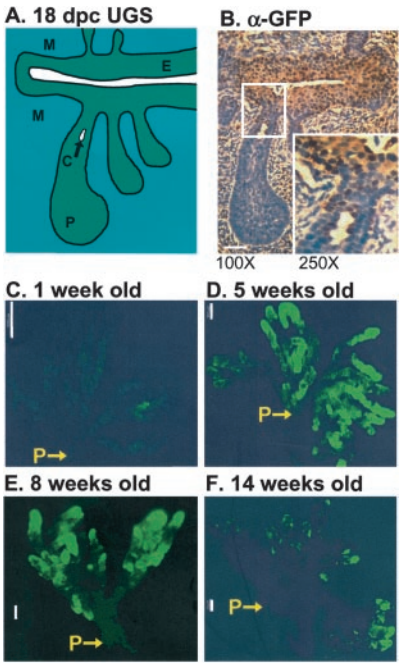

Fig. 2. GFP expression during prostate development. (*A*) Schematic diagram of a sagittal section of the male urogenital sinus at 18 dpc. M (blue), urogenital sinus mesenchyme; E (green), urogenital sinus epithelium; P (green), prostatic duct, C-proximal canalizing region of prostate. (*B*) Section of the 18 dpc transgenic urogenital sinus immunostained with anti-GFP antibody (brown) and counterstained with hematoxylin (blue). GFP expression was detected in the urogenital sinus epithelium, but not in the solid sheet of cells of the prostatic bud. Note that GFP was detected in the proximal canalizing region (magnified in the small box to the right). A serial section immunostained with control rabbit IgG showed no signal (data not shown). (*C–F*) GFP expression during postnatal development of the prostate in PSCA-GFP transgenic mice. Transillumination images of the microdissected lateral prostate from 1- (*C*), 5- (*D*), 8- (*E*), or 14-week-old (*F*) transgenic mice. Note the increasing restriction of GFP expression to the distal tips in adult mice. P, proximal end of prostate, which is connected to the urethra. (Scale bar, 200 μm.)

population of prostatic epithelial cells, which vary in position and intensity of GFP expression throughout prostatic development. Importantly, these observations suggest that at least three distinct populations of prostate epithelial cells exist. The first is a GFP-negative population of undifferentiated cells, which buds off the embryonic urogenital sinus before gland formation. The second population is GFP-positive and is associated with ductal branching morphogenesis, epithelial proliferation (growth), and differentiation (ductal canalization). The third subset consists of GFP-negative cells lining the ducts of mature glands. These cells, which comprise the majority of epithelial cells in the adult gland, are localized to intermediate regions of the ducts and are likely to be terminally differentiated.

Regulation of GFP Expression During Prostatic Involution and Regeneration. The adult prostate is an androgen-dependent gland. To study the androgen regulation of GFP and GFP-positive cells *in vivo*, we examined GFP expression in mice following castration and androgen restoration. Whereas GFP expression was detected in the distal prostatic ducts of intact 14-week-old mice (Fig. 3*A*), no expression was seen 2 weeks after castration (Fig. 3*B*). In contrast, GFP expression returned to the distal regions of the gland one day after testosterone was given (data not shown). This expression expanded and intensified as ductal regeneration continued through 1 week, then became increasingly restricted to the distal tips of maturing ducts over the next month (Fig. 3 *C* and *D*). This pattern of broad and intense GFP expression followed by increasing

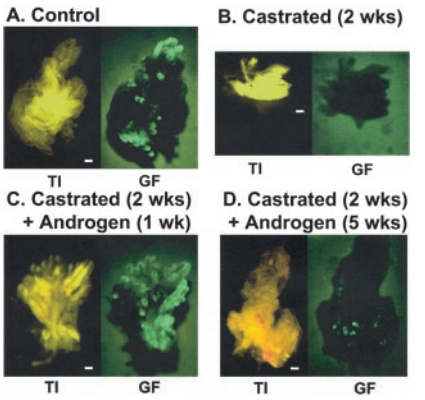

Fig. 3. GFP expression during regression and regeneration of the prostate in PSCA-GFP transgenic mice. (*A–D*) Transillumination (TI, *Left*) or green fluorescence (GF, *Right*) images of the lateral prostate from a 14-week-old intact transgenic mouse (*A*), a 14-week-old transgenic mouse castrated for two weeks (*B*), a 14-week-old transgenic mouse that had been castrated for 2 weeks and then implanted with a testosterone pellet for 1 week (*C*) or five weeks (*D*). (Scale bar, 200 μm.)

restriction to the distal tips mimics the pattern seen in the developing prostate and confirms the association of GFP expression with growth and maturation of the prostate epithelium.

Gene Expression in GFP-Positive Cells. To examine the phenotype of GFP-positive and -negative cells, the two populations were separated by flow cytometry from 8-week-old transgenic mice, processed for RNA, and analyzed for selected gene expression by reverse transcriptase (RT)-PCR (Fig. 4). β-Actin was used for normalization. GFP and endogenous PSCA mRNA expression were detected only in the GFP-positive population, confirming the integrity of the sort and the ability of the PSCA promoter to drive transgene expression exclusively in PSCA-positive cells. This result indicates that human PSCA promoter-driven expression matches endogenous mPSCA expression. Estrogen receptor β (ERβ) is expressed in the distal regions of the rat prostatic ducts (26). ERβ is detected in GFP-positive cells but not in GFP-negative cells. CD44 mRNA is detectable only in GFP-positive cells in this analysis, which is consistent with a previous report that CD44 expression in the adult mouse prostate is restricted to the ductal tips (15). ETS 2, a transcription factor implicated in prostatic development, is expressed exclusively by GFP-positive cells in the transgenic model (27). In contrast, probasin, a marker of differentiated secretory cells of the mouse prostate, was detected in both GFP-positive and -negative cells (28). These results demonstrate that

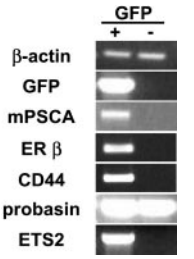

Fig. 4. Differential expression of various markers in GFP-positive and -negative cells of prostates from 8-week-old transgenic mice. GFP-positive and -negative populations were sorted for RNA preparations as described in *Materials and Methods*. Gene expression analysis of sorted cell populations for the indicated genes was performed by RT-PCR. + and − represent GFP-positive and -negative populations, respectively. ER β, estrogen receptor β.

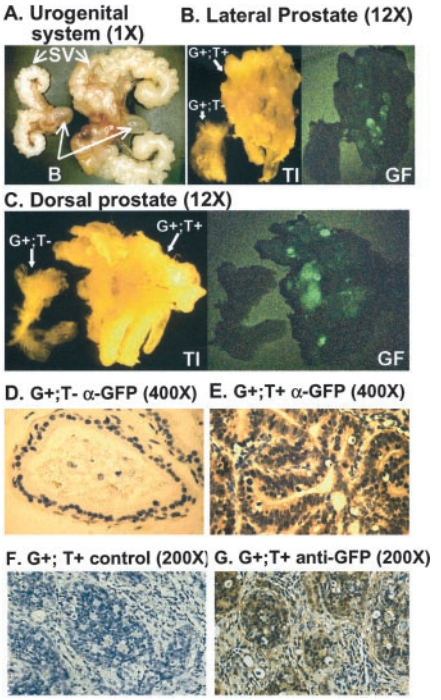

Fig. 5. GFP expression during prostate tumorigenesis in TRAMP/PSCA-GFP mice. (*A*) A urogenital system from a 23-week-old GFP+;TRAMP− transgenic mouse (*Left*) and a GFP+;TRAMP+ littermate (*Right*). SV, seminal vesicle; B, bladder. (*B* and *C*) Transillumination (TI, *Left*) or green fluorescent (GF, *Right*) images of the lateral (*B*) and dorsal (*C*) prostate from a 23-week-old GFP+;TRAMP− transgenic mouse (*Left*) or a GFP+;TRAMP+ littermate (*Right*). (*D* and *E*) Immunohistochemical staining with anti-GFP antibody of a GFP+;TRAMP− prostate (*D*) and a GFP+;TRAMP+ prostate cancer (*E*). (*F* and *G*) Immunohistochemical staining of a prostate cancer metastasis to kidney from a 23-week-old GFP+/TRAMP+ mouse with control antibody (*F*) and an anti-GFP antibody (*G*). Note the diffuse cytoplasmic expression of GFP (brown) in the TRAMP+ tumor and metastasis compared with the TRAMP-negative normal gland and the control antibody stained kidney.

GFP-positive and -negative cells differentially express markers associated with normal prostate development.

GFP Expression Is Elevated in Prostate Cancer. PSCA expression is increased in human prostate cancer and in two transgenic models of prostate cancer generated by targeted expression of T antigen (TRAMP) in the prostate and by inactivation of a single copy of the tumor suppressor PTEN (21, 23). To test the possibility that prostate cancer may arise from the cell lineage marked by GFP, PSCA-GFP mice were mated to TRAMP mice. TRAMP mice express SV40 T antigen under the control of the androgen-dependent probasin promoter. TRAMP mice develop high-grade prostate intraepithelial neoplasia (HGPIN) by 10 weeks and uniformly develop invasive prostate cancers by 28 weeks.

Female TRAMP mice were bred to PSCA-GFP males. Three litters of GFP−;TRAMP−, GFP+;TRAMP−, and GFP+;TRAMP+ male transgenics were killed at 20–25 weeks of age and examined for GFP expression. As shown in Fig. 5*A*, the prostatic lobes were dramatically enlarged and irregular in GFP+;TRAMP+, and on histologic sectioning were shown to contain invasive adenocarcinoma. Although age-matched GFP+;TRAMP− mice had little detectable GFP expression at this age, lateral and dorsal prostates of GFP+;TRAMP+ mice had moderate to strong GFP expression (Fig. 5 *B* and *C*). The strong GFP expression seen grossly with the fluorescent microscope was confirmed with immunohistochemical staining of histologic sections for GFP (Fig. 5*E*). GFP staining was not seen in the prostates of age-matched TRAMP− mice, presumably because the distal tips were not included in the section (Fig. 5*D*). In addition, a metastatic focus in kidney was identified and found to be strongly positive for GFP expression, indicating continued activity of the PSCA promoter in a prostate cancer metastasis (Fig. 5 *E* and *F*). Prostate cancers obtained from GFP−;TRAMP+ mice also did not stain positive for GFP (data not shown). All GFP+;TRAMP+ tumors examined expressed GFP. These results are similar to those reported for endogenous mPSCA in TRAMP tumors (23, 24) and demonstrate that PSCA promoter-driven GFP expression increases during tumorigenesis and metastasis.

Discussion

Association of GFP Expression with Prostate Growth and Regeneration. GFP expression in PSCA-GFP transgenic mice identifies a subpopulation of prostate epithelial cells, which is associated with periods of ductal growth during normal development and regeneration. This temporal and highly regulated expression suggests that GFP may mark a population of cells that is midway between the undifferentiated GFP-negative cells that bud off the urogenital sinus and the presumably fully differentiated GFP-negative secretory cells that line the prostatic ducts.

Similar strategies have been used to identify developmental lineages of the lung and small intestine (29, 30). Gordon and colleagues (29) generated transgenic mice that express β-galactosidase in a subset of differentiating cells during early lung development. The mouse lung arises as an outgrowth of the embryonic foregut by embryonic day 10. Reporter gene expression in these mice was detected throughout the pulmonary endoderm on 11 dpc, then extinguished in a proximal to distal wave, which parallels the order of cytodifferentiation of the pulmonary endoderm. In 16-dpc embryos, reporter gene expression was restricted to the distal region of epithelial tubules. This expression pattern is reminiscent of GFP expression in PSCA-GFP mice, suggesting that growth and differentiation of diverse epithelial ductal structures may proceed in a similar manner.

The PSCA Promoter Differs From Existing Prostate Promoters. The temporal and spatial distribution of PSCA promoter-driven GFP expression differs significantly from that reported for the rodent probasin and C3 promoters (16–18). PSCA promoter activity can be detected early in prostate development, soon after the prostate buds off the urogenital sinus. Although probasin, C3, and PSCA promoter activity are all positively regulated by androgen, the former two remain active in all secretory cells following puberty, whereas PSCA activity becomes restricted to a small population of epithelial cells in the ductal tips. Finally, probasin expression (and presumably the activity of its promoter) is lost in TRAMP tumors, whereas PSCA expression and PSCA promoter activity increase significantly in these tumors. These results suggest that the PSCA promoter can be used to target a more restricted subpopulation of prostate epithelial cells than existing prostate promoters.

Comparison of PSCA Promoter-Driven GFP with Endogenous PSCA Expression. GFP expression in PSCA-GFP mice parallels the previously reported expression of mPSCA in the mouse prostate (23, 24). Like mPSCA, GFP expression is detected in only a subset of cells and decreases with increasing age, as it becomes increasingly restricted to the distal tips of the duct. Both GFP and mPSCA are also detected in columnar cells lining the ducts. This pattern of expression differs from that reported in human prostate, in which PSCA mRNA was detected preferentially in a subset of basal cells (20). This differential pattern of expression has also been reported for CD44, which is restricted to basal cells in the human prostate and to luminal cells located in the ductal tips of the mouse prostate. Importantly, GFP expression correlates with CD44 expression in our transgenic model. One possible explanation for this discrepancy between rodent and human expression patterns is that murine and

human prostate basal cells may not be equivalent. The mouse prostate contains a smaller ratio of basal to luminal cells than the human prostate (31). Whereas 80% of proliferative activity in the human prostate is found within the basal cell layer, the reverse is true for the mouse prostate, in which most proliferative cells are located in the luminal layer. These observations suggest that the subset of basal cells that express PSCA in the human prostate could be related to the PSCA- and GFP-positive luminal cells in the mouse prostate.

The PSCA Promoter Is Regulated by Androgen. The human PSCA regulatory region is induced by androgen in both LNCaP cells and in PSCA-GFP transgenic mice. GFP expression diminished after castration and was restored on rechallenge with testosterone, in agreement with what has been reported for mPSCA expression. Similarly, PSCA promoter activity increased after exogenous addition of androgen to LNCaP cells. Analysis of a small number of deletion constructs suggests that androgen-responsive elements reside between −3 kb and −1 kb of the 9-kb fragment. It is not known whether androgen regulates the PSCA promoter directly or indirectly. It is possible, for example, that androgen induction of the PSCA promoter is related to the positive proliferative effect of androgen on LNCaP or to the expansion of GFP-positive cells during prostatic growth.

PSCA, PSCA-GFP, and Prostate Cancer. The percentage of GFP-positive cells increased dramatically in primary and metastatic TRAMP tumors. This result is in agreement with previous findings by ourselves and others showing an increased percentage of PSCA-positive cells in TRAMP tumors (23, 24). This result is also in agreement with the finding of increased PSCA expression in human prostate cancer. One possible explanation for the increase in PSCA and GFP positivity is that the PSCA promoter is activated by SV40 T antigen. However, LNCaP cells transfected with the PSCA-luciferase construct and SV40 T antigen did not show any increase in reporter gene expression (data not shown). Also, we have previously shown that murine prostate tumors caused by loss of PTEN also overexpress PSCA, suggesting that PSCA expression may be related to tumorigenesis *per se* rather than to a single initiator of transformation (23). It is also possible that the increased percentage of GFP-positive cells is related to an expansion of the cell population marked by GFP.

The functional role of PSCA in prostate carcinogenesis is not known. Other members of the Ly-6/Thy-1 gene family have been associated with increased malignant potential and metastasis (32). Antibodies against PSCA have been shown to reduce prostate tumor formation and metastasis in xenograft models (22). PSCA has also been shown recently to be overexpressed in a large percentage of bladder and pancreatic cancers (33, 34). The mechanisms governing elevated PSCA expression in these various tumor types is not known, although overexpression has been correlated with PSCA gene amplification in at least some instances (20). The results of the present study suggest that PSCA overexpression is in part a transcriptional event regulated at the level of the PSCA promoter. The transgenic model described here may be useful for the study of PSCA gene regulation in cancer.

In summary, PSCA-driven GFP expression in transgenic mice has allowed clear visualization of a subpopulation of prostatic epithelial cells associated with growth, regeneration, and transformation of the prostate. This model should provide new insights into normal and neoplastic growth of the prostate and into regulation of PSCA in cancer.

We thank the Transgenic facility at the University of California, Los Angeles, for microinjection; Dr. Norman Greenberg (Baylor College of Medicine, Houston, TX) for providing TRAMP mice; J. C. White and Shirley Quan for the preparation of the manuscript and figures; Drs. Cory Abate-Shen, Wade Bushman, Eddy deRobertis, Charles Sawyers, and Larry Zipursky for review of the manuscript; Dr. Purnima Dubey and members of the Reiter and Witte laboratories for helpful discussions; and Dr. Jonathan Said for help with histologic analysis. T.W. was a fellow of the Leukemia Society of America. O.N.W. is an Investigator of the Howard Hughes Medical Institute. This work was partially supported by Department of Defense New Idea Grant DAMD17-98-1-8578 (to R.E.R.) and grants from CapCURE (to O.N.W. and R.E.R.).

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