Noninvasive Bioluminescence Imaging of Normal and Spontaneously Transformed Prostate Tissue in Mice

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Abstract
Several transgenic mouse models of prostate cancer have been developed recently that are able to recapitulate many key biological features of the human condition. It would, therefore, be desirable to employ these models to test the efficacy of new therapeutics before clinical trial; however, the variable onset and nonvisible nature of prostate tumor development limit their use for such applications. We now report the generation of a transgenic reporter mouse that should obviate these limitations by enabling noninvasive in vivo bioluminescence imaging of normal and spontaneously transformed prostate tissue in the mouse. We used an 11-kb fragment of the human prostate-specific antigen (PSA) promoter to achieve specific and robust expression of firefly luciferase in the prostate glands of transgenic mice. Ex vivo bioluminescence imaging and in situ hybridization analysis confirmed that luciferase expression was restricted to the epithelium in all four lobes of the prostate. We also show that PSA-Luc mice exhibit decreased but readily detectable levels of in vivo bioluminescence over extended time periods following androgen ablation. These results suggest that this reporter should enable in vivo imaging of both androgen-dependent and androgen-independent prostate tumor models. As proof-of-principle, we show that we could noninvasively image SV40 T antigen–induced prostate tumorigenesis in mice with PSA-Luc. Furthermore, we show that our noninvasive imaging strategy can be successfully used to image tumor response to androgen ablation in transgenic mice and, as a result, that we can rapidly identify individual animals capable of sustaining tumor growth in the absence of androgen.

Introduction
In recent years, several transgene-based mouse models of prostate cancer have been developed that can better recapitulate many of the key molecular, physiologic, and histologic features of the human condition than conventional tumor cell–based xenograft models (1–6). These transgenic models have thus far provided significant advances in our understanding of factors that are causally implicated in the etiology, progression, maintenance, and metastasis of human prostate cancer. They should, therefore, also comprise an excellent means by which to stringently evaluate the efficacy and mechanism of new cancer treatments before their introduction in the clinic. The nonsuperficial location and often stochastic nature of spontaneous prostate tumorigenesis associated with these models significantly complicates such applications, however, as tumor burden must be estimated based on the age of the individual rather than by direct measurement. Consequently, relatively large cohorts of animals and extensive invasive end-point analyses are required to generate interpretable data.

A variety of noninvasive imaging techniques [e.g., magnetic resonance imaging (MRI), positron emission tomography, computed tomography (CT)] have recently been optimized for small laboratory animal imaging and have been used extensively to noninvasively measure tumor-specific variables in living mice (7). One such technique termed bioluminescence imaging (BLI) has many features that make it a particularly attractive approach to rapidly screen the effects of anticancer treatments with cohorts of transgenic mice. BLI relies upon the ability to detect and quantify minute quantities of light originating from luciferase-labeled cells or microorganisms, even when located at nonsuperficial locations in the living rodent. Although lacking the spatial resolution of MRI or CT imaging, this technique has been shown by us and others to be highly sensitive, and in terms of oncology research, has been used to image tumor cell metastasis and response to intervention (8, 9), spontaneous tumorigenesis (10, 11), proteasome protein–protein interactions (12–14), proteasome activity (15), apoptosis (16), and tumor cell proliferation (17) noninvasively in living mice. The ability to image the entire body of multiple animals with short acquisition times (typical range, 1-180 seconds) also confers relatively high throughput. Moreover, as only viable cells bioluminesce, this approach allows the researcher to noninvasively measure relative cell viability at regular intervals following the onset of treatment.

A prerequisite for any BLI imaging strategy is the introduction of appropriately regulated luciferase expression in the target cell (7). Therefore, in an effort to render any transgenic mouse model of prostate cancer amenable to BLI, we have generated a novel luciferase reporter mouse that strongly expresses firefly luciferase in the epithelium of all lobes of the murine prostate gland. We also present data to suggest that this reporter should enable bioluminescence imaging of androgen-independent prostate tumors in transgenic mice, as prostate tissue derived from these mice continues to bioluminesce following androgen depletion. Finally, as proof-of-principle, we show that our prostate-specific luciferase transgene can be successfully applied to image spontaneous prostate tumorigenesis, as well as tumor response to hormone ablation, in a SV40 large T and small t antigen–driven model of prostate cancer.

Materials and Methods

Generation of Transgenic Mouse
PSA-Luc mouse. As shown in Fig. 1A, the PSA-Luc transgene was constructed by positioning a large 11-kb fragment of the human
prostate-specific antigen (PSA) promoter, derived from human BAC clone CTC-771P3 (from Caltech human BAC library CTC), upstream of a firefly luciferase cDNA (derived from pGL3, Promega Corp., Madison, WI). Transgenic mice were then generated with this construct via pronuclear microinjection of FVB/N oocytes (18).

**PSA-Luc/rPB-TAg mouse.** The PSA-Luc/rPB-TAg (F26) transgenic mouse was generated via pronuclear microinjection of FVB/N oocytes with the PSA-Luc transgene (as described) and a rPB-TAg transgene, after mixing in a 1:1 molar ratio. The rPB-TAg transgene (shown in Fig. 1) comprises a 0.5-kb fragment of the rat probasin promoter, which was positioned upstream of the coding regions for SV40 large and small T antigens. These cotransgenic transgenes were found to cosegregate upon breeding, suggesting that they are stably integrated at the same chromosomal locus in F26 mice.

**Genotyping Transgenic Mice**

Following the identification of transgenic founders, transgenic mice from subsequent generations in both transgenic lines were routinely identified using luciferase primers and PCR conditions as previously described (10).

**Bioluminescence Imaging**

In vivo and ex vivo bioluminescence imaging of the transgenic mice described here was achieved using a protocol detailed previously (8, 10). IVIS Imaging System 100 and 200 series cameras, and Living Image version 2.50 software (Xenogen Corp., Alameda, CA). All animal housing and surgical procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (19).

**Histologic Analysis of Transgenic Mouse Tissues**

All tissues, except those used for in situ hybridization analysis, were fixed in 4% formalin before sectioning and H&E staining.

**In situ hybridization protocol.** Luciferase mRNA was detected in 4% paraformaldehyde-fixed prostate tissue from PSA-Luc (F488) mice using an in situ hybridization protocol described previously (20).

**cRNA probe preparation.** Firefly luciferase cRNA transcripts were synthesized from pGL3 (Promega) according to manufacturer’s conditions (Ambion, Austin, TX) and labeled with $^{35}$S-UTP (>1,000 Ci/mmol; GE Healthcare Biosciences Corp., Piscataway, NJ). cRNA transcripts >200 nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization. Sense control cRNA probes (identical to nucleotide

**Results**

**Characterization of the PSA-Luc mouse.** Both native and modified versions of the human PSA promoter have been employed by others to drive the expression of transgenes specifically to cells of the prostate gland, or cell lines of prostatic origin, in mice (21–24). Accordingly, in an effort to specifically image the prostate glands of mice noninvasively via BLI, we generated a series of PSA-Luc transgenic founder mice as described in Materials and Methods. One founder, F488, was selected for further characterization based on strong and specific in vivo bioluminescence (measured flux ~1 × 10$^9$ photons per second) arising from the lower abdomen (Fig. 2A). Ex vivo image analysis confirmed that the predominant source of this abdominal light was from the prostate glands of these mice. Furthermore, we were able to take a high-resolution image and directly visualize bioluminescence arising from all four lobes of the murine prostate gland ex vivo (Fig. 2B) by using a 40-mm field of view on an IVIS 200 series camera. Relatively weak levels (~100-fold lower) of bioluminescence were also observed from the testes and several tendons in the hind legs of founder line 488 (images not shown). Similarly, low levels of bioluminescence were also seen to originate from around the nose of PSA-Luc mice in vivo; however, the authenticity of this signal was not confirmed by ex vivo image analysis.

In the absence of a robust immunohistochemical stain for firefly luciferase on formalin-fixed tissue, we chose to employ in situ hybridization to histologically identify the cell types within the murine prostate that express luciferase mRNA. This analysis (as depicted in Fig. 2C) showed that prostatic epithelial cells from PSA-Luc (F488) mice strongly expressed luciferase. This was a critical result as our ultimate goal was to develop a bioluminescent reporter that is capable of imaging prostate carcinoma (i.e., tumors of epithelial origin) noninvasively in mice. Thus, based on measured brightness and specificity of luciferase expression, we conclude that the PSA-Luc mouse should be capable of rendering the majority of murine spontaneous prostate tumor models suitable for noninvasive optical imaging.

**Effects of androgen depletion on luciferase expression in PSA-Luc mice.** As recurrent androgen-independent prostate cancer in humans is highly refractive to existing treatments in the clinic (25), it would be highly desirable to evaluate the effects of novel cancer treatments on spontaneously developed prostate tumors in mice that have acquired the ability to grow independently of androgen. To determine whether the PSA-Luc reporter mouse could potentially enable noninvasive optical imaging of androgen-independent prostate tumors in mice, we investigated the effects of androgen depletion on bioluminescence arising from the prostates of PSA-Luc (F488) mice.

A total of 10 mice were imaged twice weekly for a period of 4 weeks (mean bioluminescence plotted as day 0 on the graph in Fig. 3B), at which point five animals were castrated. As shown in Fig. 3A and B, bioluminescence gradually decreased between 20- and 50-fold over the course of 30 days in the cohort of castrated animals relative to the intact cohort before reaching stable levels. Others have shown that transgene expression driven from the PSA promoter is highly androgen dependent (21, 26, 27); however, histologic analysis of H&E-stained tissue sections revealed that the prostate glands from the castrated cohort were severely atrophied. It is, therefore, likely that both a decrease in the number of luciferase expressing cells in the atrophied gland, along with any effects conferred by androgen depletion on reporter expression, gave rise to the observed decrease in bioluminescence from castrated mice. The fact that appreciable quantities of light could still be measured from the atrophied prostate glands of castrated mice, >1 month after androgen depletion, implies that the PSA-Luc (F488) reporter should enable in vivo BLI for other transgenic mouse models of prostate cancer following androgen ablation.
The data depicted in Fig. 3B also shows that bioluminescent intensity is stable in the noncastrated cohort of PSA-Luc mice over an extended period of time, indicating that the luciferase transgene is constitutively expressed in the prostate epithelium of these mice. The repeated BLI of intact mice in this experiment also enable us to estimate the likely sensitivity of the PSA-Luc mouse in reporting spontaneous tumor development in vivo. Light microscopy showed that the prostate glands from the noncastrated cohort of mice were histologically normal at the experimental end point (day 35 in Fig. 3B). However, the SD of mean bioluminescence for each individual PSA-Luc mouse imaged over this time period ranged from 16% to 24% (Fig. 3C). We believe this variation reflects experimental “noise” associated with our bioluminescent model rather than variability in the cellularity of the normal prostate gland. It, therefore, follows that although BLI is sensitive enough to readily detect the non-transformed prostate gland of PSA-Luc mice, we predict that it may be challenging to accurately discern subtle increases in cellularity (characteristic of the very earliest stages of tumor development) from the background bioluminescence arising from the nontransformed regions of the PSA-Luc prostate.

Figure 2. In vivo BLI (A), ex vivo BLI (B), and in situ hybridization analysis of luciferase expression (C) from the selected PSA-Luc transgenic founder (F488). Ex vivo analysis on dissected organs from PSA-Luc (F488) mice (B) confirmed that the prostate gland was the predominant source of the abdominal bioluminescent signal observed in vivo (A). These high-resolution ex vivo images (B), taken using an IVIS 200 series camera and a 40-mm field of view, show that all four lobes of the prostate gland express luciferase (SV, seminal vesicle; B, bladder; AL, anterior lobe; VL, ventral lobe; LL, lateral lobe; DL, dorsal lobe). Note that the scale bars that accompany the images in (A) and (B) display counts (or relative light units). C, results of an in situ hybridization experiment that we conducted to identify the cells within the prostate gland of the PSA-Luc (F488) mouse that express luciferase mRNA. C1 and C2, the antisense luciferase probe hybridized strongly to the epithelial cells within the prostate gland (at ×2.5 and ×10 magnification, respectively). C3 and C4, negative control and show levels of nonspecific hybridization with a complimentary sense luciferase probe (at ×2.5 and ×10 magnification, respectively).

Figure 3. A, series of in vivo bioluminescence images taken of a representative PSA-Luc (F488) mouse before and after castration (all images taken as 5-second, medium-resolution acquisitions). The scale bar that accompanies these images displays counts (or relative light units). B, graph that depicts the effects of androgen depletion on the bioluminescence of a group of individual mice [bioluminescence expressed as flux (photons/second)]. Collectively, (A) and (B) show that bioluminescence decreases between 20- and 50-fold in PSA-Luc (F488) mice following castration; however, appreciable quantities of light are still detectable using relatively short image acquisition times >1 month after castration. C, chart of measured bioluminescence of the noncastrated mice that were graphed in (B). Columns, mean; bars, SD. This variance, ranging from 16% to 24%, provides a measure of the experimental “noise” associated with this PSA-Luc mouse and bioluminescence imaging approach.
Noninvasive imaging of spontaneous prostate tumors in mice using PSA-Luc. Others have shown previously that targeted expression of SV40 large T and small t antigens to cells that comprise the murine prostate gland, via use of the rat probasin promoter, gives rise to prostate lesions that progress from hyperplasia through to high-grade lesions that eventually metastasize (1, 28). Thus, to show that our PSA-Luc transgene can be used effectively to monitor spontaneous prostate tumor development in mice via BLI, we generated transgenic founders that possessed both PSA-Luc and rPB-TAg transgenes (as depicted in Fig. 1B and described in Materials and Methods).

In vivo BLI of PSA-Luc/rPB-TAg transgenic founders identified several lines that exhibited relatively strong light emission arising specifically from the lower abdomen. As reported with other bioluminescent mouse models of spontaneous tumorigenesis (10, 11), when luciferase is regulated by a constitutively expressed promoter (shown in Fig. 3B), light emission should be proportional to the cellularity of the luciferase-expressing tissue. Thus, relative increases in bioluminescence from the lower abdomen of individual PSA-Luc/rPB-TAg mice should be indicative of spontaneous tumor development. Accordingly, individual mice from these founder lines were imaged weekly, and several of them (from which one, F26, was chosen for further analysis) indeed showed increasing amounts of bioluminescence arising from the lower abdomen over time (Fig. 4A). Ex vivo analysis (at day 90) of the F26 mouse depicted in Fig. 4A confirmed the presence of a large bioluminescent prostate tumor.

Ex vivo BLI of a cohort of PSA-Luc/rPB-TAg (F26) mice showed that the onset of this relative increase in bioluminescence varied considerably with age, ranging from 60 to 120 days (Fig. 4B). As all mice analyzed within this cohort eventually developed bioluminescent prostate tumors, these imaging results are consistent with noninvasive visualization of stochastic tumorigenesis within the cohort of PSA-Luc/rPB-TAg (F26) mice.

We next sought to investigate whether the extent of bioluminescence exhibited by F26 mice was indicative of tumor burden. A second cohort of mice (n = 9) was imaged at least thrice to establish their level of brightness, then individual mice were necropsied at various time points, so as to harvest prostate tissue that exhibited a range of bioluminescent intensities. The analysis depicted in Fig. 4B showed that bioluminescent flux ranged across two orders of magnitude from normal prostate to end-stage (palpable) tumor. Accordingly, Fig. 4C depicts three representative individuals from this cohort of nine F26 mice and shows the extent of tumor burden across these log differences in bioluminescence. Mouse #470, with a measured flux of $3.9 \times 10^8$ photons per second from the lower abdomen, was shown to have developed multiple small foci of prostatic hyperplasia. Mouse #444, however, which had a measured flux approximately one log brighter than mouse #470 ($3.4 \times 10^9$ photons per second) was shown to have developed extensive carcinoma of the prostate. Ex vivo images of this mouse also highlighted local invasion of prostatic carcinoma cells into the normally nonbioluminescent seminal vesicles. Mouse #167, which

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**Figure 4.** A, series of in vivo bioluminescent images taken at weekly intervals from a representative PSA-Luc/rPB-TAg (F26) mouse (all images shown were 10-second, high-resolution acquisitions). B, graph showing measured bioluminescent flux versus age for a group of individual PSA-Luc/rPB-TAg (F26) mice. C, in vivo and ex vivo images, as well as H&E-stained histology samples, for three individual PSA-Luc/rPB-TAg (F26) mice that exhibit approximately log differences in measured in vivo bioluminescent flux. Mouse #470, which exhibits a measured flux of $3.9 \times 10^8$ photons per second, has essentially a normal prostate with multiple regions of epithelial hyperplasia (white arrows). Mouse #444, with a measured flux $\approx 10$-fold higher, had developed large areas of poorly differentiated prostate carcinoma and local invasion into the otherwise nonbioluminescent seminal vesicles. Mouse #167 has a measured flux approximately one log brighter still and has developed a large, palpable, and necrotic (as evidenced histologically by lightly H&E-stained regions) prostate tumor. This mouse also developed, independently of the primary prostate tumor, a bioluminescent thymoma. Note that the length of the yellow bars associated with each ex vivo image corresponds to 1 cm.
Bioluminescence imaging of spontaneous prostate tumor response to androgen depletion. Based on the relationship between measured in vivo bioluminescence and tumor burden, we determined that a flux of \(1 \times 10^9\) photons per second was indicative of substantial tumor burden (1 cm tumor diameter) in F26 mice (Fig. 5A). To investigate whether our PSA-Luc transgene could be used to noninvasively image spontaneously arisen prostate tumors in androgen-depleted animals, we castrated five individual mice once they had spontaneously reached a level of brightness of \(1 \times 10^9\) photons per second. Thereafter, the effect of androgen depletion on bioluminescence was determined by imaging all mice on a weekly basis.

The results of this experiment (Fig. 5B) showed that two from five individuals became increasingly bright over time and had to be euthanized 4 weeks post-castration after developing large palpable prostate tumors. Three from five individuals, however, exhibited a gradual decrease in bioluminescence over a similar time period, eventually reaching a stable level \(100\)-fold lower than when castrated. These individuals continued to bioluminesce at approximately this level of brightness over the next 5 months while remaining ostensibly healthy. Ex vivo analysis confirmed that these individual mice exhibiting relatively low levels of bioluminescence were tumor free. Necropsy also showed that the remaining prostate tissue was severely atrophied in these mice, and that the seminal vesicles were no longer macroscopically visible. The tumor response to androgen ablation observed here is consistent with that reported previously for the original TRAMP (rat prostatein regulated SV40 large T and small t antigen) mouse, whereby \(50\%\) of individual mice were shown to be capable of developing tumors after castration (28, 29). The results of this experiment also show that the imaging strategy we employ here enables rapid identification of individual mice with tumors that are resistant to the effects of androgen ablation. Within 10 days of castration, we were able to measure more than a log difference in bioluminescence between groups of individual mice with prostate tumors that ultimately proved resistant or susceptible to the effects of androgen ablation.

Discussion

We have used a large fragment of human PSA promoter to generate a luciferase reporter mouse that, due to the specificity and intensity of bioluminescence, should enable in vivo BLI of any preexisting transgenic mouse model of prostate cancer. As proof-of-principle, we used this PSA-Luc reporter transgene to generate another transgenic mouse that developed bioluminescent SV40 large T and small t antigen–driven prostate tumors in a manner similar to the TRAMP mouse. This experiment provided good validation of our imaging strategy, as because both luciferase reporter and oncogene are transcriptionally regulated by different prostate-specific promoters, it essentially recapitulates how others would employ our PSA-Luc (F488) reporter mouse to optically image their prostate tumor model of interest.

Several other reports have been published recently that describe a variety of transgenic strategies that should facilitate BLI of spontaneous prostate tumors in mice (27, 30–32). However, to date, no group has directly shown either the success or limitations of their approach by applying it to image spontaneously arisen prostate tumors in transgenic mice. Of these, the EZC-prostate mouse [using a human kallikrein 2 (hK2)–based promoter] was generated to express both firefly luciferase and eGFP in the murine prostate and thus be amenable to both bioluminescence and fluorescence imaging (30). The characterization of this mouse showed weak or undetectable reporter expression in the dorsolateral and anterior lobes of the murine prostate, respectively, which implies that the EZC-prostate mouse may not enable in vivo BLI for all murine prostate tumor models. Moreover, the sensitivity of this approach was reduced by significant levels of background bioluminescence arising from the cecum of these mice. In contrast, we show that the PSA-Luc (F488) reporter mouse exhibits robust bioluminescence from all four lobes of the murine prostate, with negligible nonspecific light emission arising from proximal nonprostate tissues. The other reported approaches have used a transgenic approach termed two-step transcriptional amplification.

had a measured flux approximately one log brighter still \((2.4 \times 10^{10}\) photons per second), had developed a large palpable tumor, although otherwise seemed healthy and asymptomatic. Ex vivo imaging of this tumor highlighted marked heterogeneity in the intensity of emitted light, which is likely attributable to the extensive necrosis observed at the histologic level.

Other F26 mice with end-stage primary prostate tumors \((>1 \times 10^9\) photons per second) also highlighted frequent bioluminescent metastases to pelvic lymph nodes and lungs, which although not evident from in vivo imaging, could be readily detected ex vivo at necropsy and were confirmed by histology (data not shown). A subset of older F26 mice was also seen to develop bioluminescent thymomas independently of and with a relatively longer latency than the primary tumors developing in the prostate (as illustrated with mouse 167 in Fig. 4C).
(TSTA), with overall prostate specificity of transgene expression governed by the use of either the PSA promoter (33) or the PSE promoter (a modified form of the human PSA promoter; ref. 22). This bitransgeneic approach was deemed necessary because tissue-specific promoters are seldom as strong as viral promoters. Although true, we show here that luciferase transgene expression regulated by a larger 11-kb portion of the PSA promoter is in itself sufficient to enable easy visualization of nontransformed prostate tissue in transgenic mice via BLI with relatively short image acquisition times (~10 seconds). Others have previously shown that a longer version of the probasin promoter (~11 kb) also gave rise to higher levels of transgene expression in the prostate glands of mice than the minimal 0.5-kb form (3, 34). The ability of these larger promoter fragments to drive higher levels of tissue-specific transgene expression relative to their shorter forms may be due to the presence of additional transcriptional regulatory elements that are not present in the short promoter sequence. In support of this notion, we observed similarly high levels of bioluminescent intensity from several of the other PSA-Luc and rPB-TAg/PSA-Luc transgenic founder lines that were not selected for further analysis (data not shown). This observation suggests that transgene copy number and the site of transgene integration, which both vary between founder lines, may play a lesser role in generating the high levels of bioluminescence that we observed in our transgenic mice. A disadvantage of using longer promoter fragments to drive transgene expression versus the tissue-specific TSTA approach, however, is that often the final transgene is too large to be delivered by adenoviral or lentiviral vectors to somatic cells in vivo (31, 35).

An important aspect of human prostate cancer that several transgenic tumor models are able to recapitulate is the development of tumors that can grow independently of androgen. As androgen-independent prostate cancer is associated with poor clinical prognosis, it would be highly desirable to develop a luciferase reporter that could facilitate drug development via BLI with androgen-independent transgenic tumor models too. We show that castrated PSA-Luc (F488) mice exhibit a steady decrease in in vivo bioluminescence over a period of 4 weeks, eventually reaching stable levels that were between 2% and 5% as bright as the noncastrated cohort. Despite this decrease, we show that castrated PSA-Luc (F488) mice retain significant amounts of prostate-specific bioluminescence in the presence of castrate levels of androgen. This suggests that these mice should enable BLI for both androgen-dependent and androgen-independent transgenic prostate tumor models in vivo.

Others have shown previously that transgene expression regulated by a shorter length of PSA promoter is highly dependent upon androgen and have reported a similar degree of reduced expression within a week of castration and even the complete loss of expression by 23 days (21, 27). The rate at which transgene expression decreased in the castrated cohort of PSA-Luc (F488) mice seems to be different and slower than these other published studies. We also found that severe atrophy of normal prostate tissue in these mice contributed significantly to the overall decrease in measured in vivo bioluminescence.

The results of a longitudinal BLI study on castrated tumor-bearing PSA-Luc/rPB-TAg (F26) mice also provided experimental evidence to support the suggestion that the PSA-Luc (F488) mouse will enable in vivo BLI of transgenic prostate tumors irrespective of androgen. This experiment showed that these mice can be used to follow the response of a tumor to androgen ablation over extended periods of time. Moreover, these results showed that it was possible to readily identify tumors in mice that would ultimately prove resistant or susceptible to the effects of androgen deprivation soon after castration. The ability to do this affords an opportunity, lasting several weeks, to evaluate the efficacy of novel treatments on spontaneously arisen androgen-independent prostate tumors in mice.

Despite the relatively small cohort size, these results essentially recapitulate the tumor phenotype associated with the nonbioluminescent TRAMP mouse. It is likely that a similar mutagenic event has occurred in those mice that developed the androgen-independent tumors. Our data also suggest that such a secondary mutation is likely to have arisen early in the development of these tumors, as we did not detect an extensive decrease in bioluminescence, which reflects cell viability, before androgen-independent tumor growth.

In conclusion, we describe here the generation of a set of luciferase reporter mice that can noninvasively image normal and spontaneously transformed prostate tissue in mice, in both the presence and absence of androgen. We envisage that the main application of these reporter mice will be to facilitate the use of various transgenic prostate tumor models in drug development. Achieving this goal will directly improve the stringency of preclinical drug trials, which in turn should improve the quality of treatments that enter the clinic.

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References

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