Androgen-Independent Molecular Imaging Vectors to Detect Castration-Resistant and Metastatic Prostate Cancer

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Abstract

Prostate-specific promoters are frequently employed in gene-mediated molecular imaging and therapeutic vectors to diagnose and treat castration-resistant prostate cancer (CRPC) that emerges from hormone ablation therapy. Many of the conventional prostate-specific promoters rely on the androgen axis to drive gene expression. However, considering the cancer heterogeneity and varying androgen receptor status, we herein evaluated the utility of prostate-specific enhancing sequence (PSES), an androgen-independent promoter in CRPC. The PSES is a fused enhancer derived from the prostate-specific antigen (PSA) and prostate-specific membrane antigen gene regulatory region. We augmented the activity of PSES by the two-step transcriptional amplification (TSTA) system to drive the expression of imaging reporter genes for either bioluminescent or positron emission tomography (PET) imaging. The engineered PSES–TSTA system exhibits greatly elevated transcriptional activity, androgen independency, and strong prostate specificity, verified in cell culture and preclinical animal experiments. These advantageous features of PSES–TSTA elicit superior gene expression capability for CRPC in comparison with the androgen-dependent PSA promoter–driven system. In preclinical settings, we showed robust PET imaging capacity of PSES–TSTA in a castrated prostate xenograft model. Moreover, intravenous administrated PSES–TSTA bioluminescent vector correctly identified tibial bone marrow metastases in 9 of 9 animals, whereas NaF- and FDG-PET was unable to detect the lesions. Taken together, this study showed the promising utility of a potent, androgen-independent, and prostate cancer–specific expression system in directing gene-based molecular imaging in CRPC, even in the context of androgen deprivation therapy.

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Introduction

Prostate cancer is the most common cancer for males in America. The disseminated disease remains a major cause of cancer-related morbidity and mortality (1). Hormone ablation or androgen deprivation therapy (ADT) is so far the most effective systemic treatment for patients with metastasis (2). However, despite initial response to androgen withdrawal, castration-resistant prostate cancer (CRPC) progression will occur within an average of 12 to 18 months in the majority of the cases (1). Due to the unfortunate lack of cure for CRPC, the search for more effective treatment and diagnostic regimen deserves urgent attention.

Adenovirus (Ad)-mediated molecular imaging and gene therapy vectors have been intensely studied to diagnose and treat CRPC for the past decade (ref. 3, and reviewed by ref. 4). In these Ad vectors, prostate-specific promoters or enhancers such as the ones derived from prostate-specific antigen (PSA), probasin, and human glandular kallikrein 2 (hK2) have been broadly used to drive tissue-restricted transgene expression, so as to ensure the minimum toxicity to normal and nontargeted organs (3). Many of these promoters rely on the presence of testosterone and activated androgen receptor (AR) for transcription. A large volume of evidence has shown that AR remains active in CRPC via mechanisms such as AR amplification, mutation or intragenic rearrangement, upregulation of coactivators, ligand-independent activation of AR, as well as emergence of hyperactive AR splicing variants (5–8), theoretically supporting the use of AR-driven promoters in castrated patients. However, prostate cancer exhibits great heterogeneity (9) and the functional status of AR vary amongst different metastatic lesions and primary tumors (10). Moreover, under acute maximal ADT, transcriptional activity of AR is expected to be strongly inhibited. A prostate cancer selective but less androgen- or AR-dependent promoter would likely be more active under this setting. Interestingly, the transcriptional regulation of the prostate-specific membrane antigen (PSMA) gene differs dramatically from the PSA gene in that it is negatively regulated by androgen (i.e., androgen...
suppressive; refs. 11, 12). In addition to its proximal 1.2-kb promoter of PSMA, the prostate specificity and androgen-suppressive activity is highly regulated by its enhancer element (PSME), located in the third intron of the FOLH1 gene (13). The expression of PSMA was found to be elevated in more malignant prostate cancer, CRPC, as well as tumor-associated vasculature (14). Although the transcriptional regulatory mechanism of PSME is still not well understood, several groups have exploited the strong prostate cancer selectivity of PSMA promoter/enhancer (PSMAP/E) to direct gene therapy against advanced prostate cancer (15, 16). Kao and colleagues have further advanced the prostate cancer-selective gene expression strategy by creating a chimeric prostate-specific enhancing sequence (PSES; ref. 17) that is comprised of gene regulatory elements from both the androgen-inducible PSA enhancer and the androgen-suppressive PSMA enhancer. Consequently, PSES can activate gene expression irrespectively of androgen status, making it a promising promoter for gene therapy in subjects with CRPC, especially considering the scenario of combining gene therapy under maximal ADT. The activity of PSES, however, is relatively limited compared with constitutive promoters (17).

In this study, we took advantage of the 2-step transcriptional amplification (TSTA) system to boost the transgene expression level of PSES. The principle of TSTA system is shown in Figure 1A. In the first step, PSES drives the expression of the chimeric activator GAL4-VP16 (ref. 18; GAL4 is the DNA-binding domain whereas VP16 domain activates transcription), which then augments the transcription of the reporter genes upon recruitment to 5 GAL4 binding sites (G5). Two Ad vectors were generated with PSES-TSTA system regulating the expression of firefly luciferase (FL) or a variant of herpes simplex virus-thymidine kinase (HSV-sr39tk) as bioluminescent and PET imaging reporter gene, respectively.

Materials and Methods

Adenoviral generation

Adenoviral vectors were constructed on the basis of a modified AdEasy system—the AdNUEZ system, in which transgenes can be placed into the E3 region by multiple cloning sites. In Ad-PSES-TSTA-FL, the PSES-GAL4VP16 was constructed by replacing PSEBC with PSES in pBnewVP2BS. PSES-GAL4VP16 was then cloned into pAdNUEZ vector to generate pAd-PSES-VP2EZ. pShuttleG5-FL was then used for recombination with pAd-PSES-VP2EZ. In Ad-PSES-TSTA-DAbR1-sr39tk, the E3 region was constructed by subcloning G5-sr39tk into pNEB193, followed by insertion of PSES-GAL4VP16 in tail-to-tail configuration. The 2 cassettes were then cut out by SpeI and placed into pAdNUEZ to generate pAd-G5sr39tkPSESVP2EZ. pShuttleG5-DAbR1 was constructed by replacing FL with DAbR1 in pShuttleG5-FL. Homologous recombination of pAdEZ and pShuttle was realized in E. Coli BJ5183-competent cells. Viral clones were screened, propagated, purified, and titered as previously described (19).

Cell lines and cell culture experiments

All cell lines were cultured in medium containing 10% FBS and 1% penicillin/streptomycin. The CWR22Rv1, LNCaP, C4-2, HeLa, and A549 cells were maintained in RPMI1640. EMEM was used for DU145 cells. The VCaP, PaCa-2, and MDA-MB-231 cells were grown in Dulbecco's modified Eagle's medium. The LAPC-4 and LAPC-9 models were authenticated by UCLA Chancellor's Animal Research Committee for the absence of Mycoplasma. The VCaP cells were a kind gift from Dr. Robert Reiter. The other cell lines were obtained from AACR and were not further tested or...
authenticated. All cell culture experiments were conducted with cells at less than 35 passages after receipt. Synthetic androgen methylenetrionelone (R1881; NEN Life Science Products) was used at 10 nmol/L. AR antagonist bicalutamide and MDV3100 was added to media as indicated at 10 μmol/L.

For in vitro luciferase assay, cells were seeded onto 24-well plates at 5 × 10^4 cells per well and infected the next day. All in vitro infection was done with multiplicity of infection (MOI) of 1. At 72 hours postinfection, the cells were harvested and lysed in passive lysis buffer (Promega). FL luciferase activity was measured according to the manufacturer’s instructions (Promega) using a luminometer (Berthold Detection Systems). Each value was normalized to cell number or protein amount and calculated as the average of triplicate samples. The activity was then normalized to that of Ad-PSES-TSTA-FL infected CWR22Rv1 cells cultured in R1881 condition so that different experiments can be compared across. Due to the similarity of infectivity among human cell lines, activity results were not adjusted.

For Western blot, 5 × 10^5 CWR22Rv1 and LNCaP cells were seeded into each well in 6-well plates and infected with indicated virus the next day. Seventy-two hours postinfection, cells were collected and lysed in passive lysis buffer, and cell lysates were fractionated on 4% to 12% gradient acrylamide gels (Invitrogen) and subjected to immunoblot analysis using monoclonal anti–HSV-tk antibody kindly provided by Dr. Margaret Black, polyclonal anti-human Fc antibody (Jackson Immunoresearch Laboratories, Inc.) and monoclonal anti–β-actin A5316 antibody (Sigma Chemical Co.). Visualization was done by BM Chemiluminescence (Roche Diagnostics) with horseradish peroxidase–conjugated respective antibodies (Santa Cruz Biotechnology).

**Subcutaneous tumor xenograft experiments**

Animal experiments were done in accordance with the University of California Animal Research Committee guidelines. A total of 5 × 10^6 CWR22Rv1 cells that were marked with lentivirus expressing CMV-driven Renilla luciferase were implanted subcutaneously onto both flanks of 4- to 6-week-old female severe combined immunodeficiency (SCID) mice (Taconic Farms) in Matrigel (1:1 v/v; BD Biosciences). A total of 1 × 10^7 plaque forming units (PFU) viruses were intratumorally injected. Luciferase expression was monitored using IVIS cooled CCD camera (Xenogen). Images were analyzed according to previously described procedures (19). Animals and dissecting the indicated organs. Luciferase imaging and analysis were done as described above.

**PET imaging experiment**

Subcutaneous LAPC-4 AI was serial passaged in castrated SCID-Beige mice (Taconic Farms). Tumor chunks at the size of (3 mm)^3 were cut and implanted subcutaneously onto the right shoulder of castrated SCID-Beige mice. Five weeks later, a total of 1 × 10^7 to 2 × 10^6 PFU Ad-PSES-TSTA-sr39tk-DAbR1 was injected intratumorally in 4 consecutive days. Seven days after the initial infection, 18F-FHBG PET imaging was done according to previously described procedures (19). Animals were sacrificed after imaging.

**Intratibial tumor experiment**

Four- to 6-week-old male SCID-Beige mice (Taconic Farms) were castrated by orchietomy according to UCLA ARC procedure. Two weeks later, 1 × 10^5 LAPC-4 AI (20) tumor cells were injected into the right tibial bone marrow in Matrigel from underneath the knee. Six weeks later, the animals received 1 × 10^6 PFU of Ad-CMV-GFP and 4 × 10^8 PFU Ad-PSES-TSTA-FL virus through tail vein with a 4-hour interval in between. The purpose of predosing with irrelevant CMV-GFP virus is to blunt the Kuffer cells in the liver to improve transduction efficiency (21). FL-mediated BLI was done 4 days postviral administration. 18F-NaF and 18F-FDG PET imaging was done 6 and 11 days postinfection, respectively. Briefly, for both 18F-NaF and 18F-FDG PET imaging, 70 μCi of probe was injected through the tail vein or ip., respectively, and the animals were allowed to move around and excrete during 1-hour probe uptake. Afterwards, animals were given inhalation isoflurane anesthesia, placed in a prone position and imaged for 10 minutes in the microPET scanner. A 10-minute CAT imaging session followed to provide structural information. All animals were sacrificed after the last imaging session, and tibial bone from both sides were removed and subjected to immunohistologic staining with anti–pan-cytokeratin antibody (Bio-Genex).

**Statistical analysis**

Statistical analyses were done using the 1-tailed Mann–Whitney Test. For all analyses, P < 0.05 was considered statistically significant.

**Results**

**PSES–TSTA activates gene expression under androgen deprivation or AR blockade**

To examine the cell-targeted transcriptional activity of the PSES–TSTA system, we constructed 2 recombinant adenoviral vectors (Ads) that control the expression of the imaging reporter gene FL or HSV-tk39tk, respectively (Fig. 1B). Recapturing extensive prior experiences (18, 22, 23), the incorporation of TSTA system dramatically boosted the promoter activity of PSES by at least 3 orders of magnitude compared with the parental PSES straight vector (Supplementary Fig. S1A). Another advantageous feature of the TSTA system is its ability to drive the expression of multiple functional
transgenes in the same vector simultaneously, exemplified by the Ad-PSES-TSTA-DAbR1-sr39tk vector (Fig. 1B). The DAbR1 gene (DOTA Antibody Reporter 1) encodes a membrane-anchored engineered antibody that can irreversibly bind to radiometal chelates (24). Therefore, similar to HSV-sr39tk, it possesses both imaging and cytotoxic therapeutic capability. We have confirmed the expression of DAbR1 in Ad-PSES-TSTA-DAbR1-sr39tk (Supplementary Fig. S1B). However, we did not explore the utility of DAbR1 here as we are actively investigating the multimodal imaging, suicide, and radioimmunotherapeutic capability of Ad-PSES-TSTA-DAbR1-sr39tk in a comprehensive treatment study of CRPC.

Next, we thoroughly evaluated the androgen responsiveness of Ad-PSES-TSTA-FL in comparison with the enhanced PSA promoter–driven Ad-PSA-TSTA-FL (previously denoted as AdTSTA-FL; ref. 18) in androgen-dependent (AD) cell line LNCaP (Fig. 2A), AR expressing yet androgen-independent (AI) CWR22Rv1, VCaP and C4-2 (Fig. 2B), as well as AR-negative prostate cancer cell line DU145 (Fig. 2C). AR expression was verified in Supplementary Figure S2A. To investigate the magnitude of androgen induction, the vector-infected cells were cultured in media supplemented with either the synthetic androgen R1881 or AR antagonists. Both the first-generation agent bicalutamide and the more potent second-generation antagonist MDV3100 (25) were examined. In the highly AR-dependent, AD cell line LNCaP, bicalutamide and MDV3100 treatment substantially reduced PSA–TSTA–driven FL expression to 0.6% and 0.2%, respectively, compared with the R1881 condition, whereas the PSES–TSTA–driven FL only dropped down to 13% and 10%. In contrast, in the AI cell line CWR22Rv1, the presence of bicalutamide and MDV3100 decreased PSA-TSTA-FL activity to 28% and 32% of the R1881 condition, but the PSES–TSTA–driven FL expression was upregulated to 134% and 136%, respectively. Consistent results were observed in VCaP and C4-2 cells as well. In the DU145 cells that lack AR, both PSA-TSTA and PSES–TSTA vector were inactive (Fig. 2C). Remarkably, the absolute FL activity from the PSES–TSTA promoter was higher than that from PSA–TSTA in all the conditions tested in the AR expressing tumor cells. The results in the DU145 cell line support that AR is necessary for the androgen-suppressible activity of PSME.

Next, the gene expression capabilities of our vectors were examined in tumor-bearing animals. In our initial experiment, we implanted 2 CWR22Rv1 xenografts, stably expressing Renilla luciferase (RL), onto both flanks of female SCID mice (n = 9), using the low androgen level in female animals to mimic an androgen-deprived condition. The RL-mediated BLI...
verified the establishment of both tumors that grew to similar volume (Fig. 2D, left panel). Subsequently, equivalent doses of Ad-PSA-TSTA-FL and Ad-PSES-TSTA-FL vector were injected into the right and left tumor, respectively. In vivo FL-imaging verified the PSES–TSTA vector is about 10-fold more potent than the PSA–TSTA vector in CWR22Rv1 tumors in androgen-deficient conditions (Fig. 2D). To further substantiate the superior activity of PSES vector in an androgen-deprived setting, we injected Ad-PSES-TSTA-FL or Ad-PSA-TSTA-FL into solitary LAPC-9 AI (androgen-independent subline of LAPC-9) tumors established in castrated male SCID-Beige mice (20). The intratumoral FL signal directed by the PSES-driven vector was nearly 100-fold higher than the PSA-driven vector (Supplementary Fig. S2B and C). In summary, these results showed that PSES–TSTA remained transcriptionally active regardless of androgen status. The Ad-PSES-TSTA-FL was able to achieve 10- to 100-fold higher gene expression level than the androgen-dependent PSA–TSTA–driven vector in several CRPC models.

**PSES–TSTA retains stringent prostate tissue selectivity**

The prostate specificity of PSES has been documented in previous reports (17). However, it is necessary to evaluate the specificity of the amplified PSES–TSTA system, considering the introduction of the strong GAL4-VP16 transcriptional activator. First, a panel of cancer cell lines from various tissue origins was transduced with Ad-PSES-TSTA-FL. Robust expression was detected only in the prostate cancer cell line CWR22Rv1, whereas PSES–TSTA expression was restricted in nonprostatic cancer cell lines (Fig. 3A).

To confirm the tissue specificity in vivo, we injected Ad-PSES-TSTA-FL into the prostate of male SCID mice (n = 5). The control cohort received the universal CMV promoter-driven vector. In vivo BLI was done 7 and 14 days afterwards (Fig. 3B and C). Similar to our previous studies (26), we observed the systemic vector leakage despite the orthotopic injection manifested by the strong liver signal displayed from the CMV group. In contrast, Ad-PSES-TSTA-FL activated gene expression selectively in the prostate gland while inhibiting expression in the liver. Ex vivo imaging of harvested liver, testicles, and prostate seminal vesicles (PSV) again confirmed the prostate specificity of PSES–TSTA seen in vivo (Fig. 3D). Collectively, our results showed that the PSES–TSTA system activated gene expression only in prostate cancer cell lines and that the in vivo transcriptional activity was restricted to the prostate.

**Directing PET imaging in castration-resistant prostate cancer**

BLI has very limited application as a whole-body imaging modality in clinical settings due to its poor tissue penetration and inability to provide quantitative signal in vivo. Previous experience with PET reporter genes such as HSV-sr39tk indicated that it is less sensitive than bioluminescent reporter genes and, therefore, demands a very high level of gene expression (19). Encouraged by the amplified activity of PSES–TSTA, we explored its ability to direct PET imaging in CRPC tumors. We first examined the magnitude of \( HSV\text{-}sr39tk \) gene product expressed by Ad-PSES-TSTA-DABR1-sr39tk (Fig. 1B) in the LNCaP and CWR22Rv1 prostate cancer cell lines (Fig. 4A). Corroborating the previous result of the Ad-PSES-TSTA-FL vector (Fig. 2A and B), we observed that the PSES–TSTA is capable of achieving not only androgen-independent expression compared with PSA–TSTA but also very robust expression of reporter gene, exceeding even the level achieved by the strong universal CMV vector.

Next, we examined the in vivo PET imaging capability of Ad-PSES-TSTA-DABR1-sr39tk with the use of the PET reporter probe 9- \( \text{[18F]-fluoro-3-hydroxymethylbutyl} \) guanine (\( \text{[18F-FHBG} \)) in an androgen-independent subline of LAPC-4 prostate cancer cells (LAPC-4 AI) xenografts (20). As shown in Figure 4B and C, intratumoral delivery of \( 1 \times 10^9 \) to \( 1.5 \times 10^9 \) PFU of Ad-PSES-TSTA-DABR1-sr39tk was able to produce distinct tumor signal in all 3 castrated animals that were tested. The asterisks indicated accumulation of circulating probe in the cardiac blood pool, and it is not attributed to nonspecific gene expression from the PSES–TSTA system. Overall, these results support the feasibility of PSES–TSTA to direct tumor-specific PET imaging in CRPC patients, even in the setting of ADT.

**Image bony prostate cancer metastasis with systemic vector administration**

Bone is the major site for prostate cancer metastasis (27, 28). Osseous involvement of this disease severely worsens the prognosis and quality of life. A reliable diagnostic tool to detect emergence of bony metastasis would be, therefore, highly valuable. To investigate the applicability of the PSES–TSTA in detecting bone metastasis, we injected LAPC-4 AI cells directly into the right tibia of 9 castrated SCID-Beige mice, to develop osseous lesions (29). As shown in the timeline of this study (Fig. 5A), Ad-PSES-TSTA-FL was administered through the tail vein 6 weeks after tumor cell implantation. This time point was chosen on the basis of our previous experience showing that, although there is some variability in the rate of tumor establishment with this model, a majority of osseous lesions will be established in this time frame. In vivo BLI detected positive FL signals in the right tibia in 7 of the 9 animals (Fig. 5B). Remarkably, immunohistochemistry staining with a human epithelial cell-specific marker, pan-cytokeratin, showed that the 2 negative animals indeed failed to establish any tumor in the bone (Fig. 5C). With the other hand, the presence of prostate cancer in the 7 "positive" animals was confirmed by either pan-cytokeratin staining or gross observation of the bone (Fig. 5C and Supplementary Fig. S3). Furthermore, this imaging approach qualitatively reflects the volume of tumor mass because the magnitude of FL luminescence roughly correlated with the intensity of cytokeratin staining of the tumor mass (Fig. 5C). Importantly, we observed no emission at any other site, albeit a negligible level in the liver of one animal, showing the high prostate cancer specificity of PSES–TSTA (Fig. 5B).

Next, we decided to compare the PSES–TSTA approach to current clinical PET-based imaging technologies. We assessed the diagnostic ability of 2 conventional PET tracers \( \text{[18F-FDG} \) to image these animals bearing

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osseous lesions 2 and 7 days after the BLI (Fig. 5A). The fluoride ion tends to deposit at active bone remodeling sites, such as cancer osseous metastatic lesions, where intertwined interactions of cancer cells, osteoblasts, and osteoclasts dynamically occur. Thus, there is an increased interest to use 18F-NaF as a tracer to assess metastatic bone lesions for prostate and other cancerous malignancies (29, 30). However, as shown in the left panel of Figure 5D, 18F-NaF PET-CT failed to identify the tumor-bearing tibia in the 6 animals examined. On the other hand, due to the well known Warburg effect of heightened glucose metabolism in most cancer, 18F-FDG has been used as the standard PET imaging tracer in oncology (29, 31). Hence, we also assessed the detection capability of 18F-FDG PET-CT for bone metastases (Fig. 5D, right panel). Out of the 6 animals examined, 18F-FDG PET imaging was unable to distinguish between animals with histologically proven lesions from those without lesions. Nor could 18F-FDG distinguish between the lesion-positive (right) and -negative (left) tibia within the same animal.

We concluded that systemically injected Ad-PSES-TSTA-FL and subsequent BLI can detect an experimental prostate cancer osseous metastasis model with high degree of accuracy (9 out of 9 animals). This type of transcription-based imaging could be more specific than 18F-FDG and 18F-NaF PET-CT, 2 standard methods currently used in clinics.

Discussion

In the battle against advanced prostate cancer, there is an urgent and unmet need for a selective imaging modality. The transcription-based molecular imaging approach is particularly advantageous in its ability to be tailored to the molecular and genetic alterations in cancer (32, 33). Recent preclinical and clinical evidence show that AR signaling is required to sustain the growth of prostate cancer, even in CRPC (5–9, 34). On the basis of this property, we and others employed the AR-dependent PSA promoter to drive reporter gene–based imaging and showed the feasibility of this
approach to monitor dynamic AR transcription function in vivo, to image CRPC and metastatic diseases (33, 35–37). Although the TSTA system can boost the activity of the PSA promoter and facilitate imaging of CRPC tumors, we speculate that the incorporation of an androgen-independent and cancer-selective gene regulatory element could be more efficacious in driving the imaging reporter, especially in the context of maximal androgen blockade therapy. To this end, the enhancer element of the PSMA gene (PSME) seemed to be particularly promising due to its prostate cancer specificity and its unique androgen-suppressible transcriptional activity (12). In this study, we applied the TSTA amplification to the PSES promoter, which fused the PSME with the PSA enhancer element. The resulting PSES–TSTA vectors were able to achieve dramatically elevated transcriptional potency compared with the parental PSES promoter alone. Moreover, they were capable of directing androgen-independent and prostate cancer–specific expression. As a consequence of their augmented capabilities, we observed that the utility of the PSES–TSTA vector in imaging application for CRPC was also expanded. For instance, we showed that the PSES–TSTA vector expressing the HSV-sr39tk gene elicits robust PET signals in CRPC tumors. Moreover, systemic delivery of Ad-PSES-TSTA-FL and subsequent BLI was capable of detecting bony CRPC metastases in a manner more sensitive and specific than current, conventional PET imaging modalities. The ability of the PSES–TSTA vector to accomplish this challenging task of detecting metastatic lesions within the systemic circulation further affirms the CRPC-selective expression capability of the PSES–TSTA vector.

At this current juncture, the mechanism of the intriguing androgen-suppressible activity of PSME is not well understood. It is clear that linkage of PSME to the PSA enhancer countered the highly androgen/AR-induced activity of the PSA enhancer, especially in the context of acute AR blockade (Fig. 2). In response to treatment with AR antagonists, the activity of the PSA enhancer–driven reporter (Ad-PSA-TSTA-FL) was drastically inhibited in both AD LNCaP cell line and castration-resistant AR-positive cell lines. Moreover, the magnitude of PSA–TSTA activity suppression correlated with the potency of AR antagonists (Fig. 2A), as the second generation antagonist MDV3100 was reported to be nearly 10-fold more active than bicalutamide, the first generation agent (25). In contrast, the PSES–TSTA–mediated luciferase signal was not sensitive to AR blockade, even under the more efficacious MDV3100 treatment. The activity of the PSA enhancer component of PSES can be appreciated in highly androgen- and AR-dependent settings. For instance, in LNCaP cells in the presence of androgen, the activity of PSA–TSTA vector is almost equivalent to the PSES–TSTA vector. However, in CRPC models, despite ample level of androgen, the expression level of
PSA–TSTA remains significantly reduced (by more than 100-fold) when compared with PSES–TSTA. This result again highlights that, in the context of suppressed AR function, the contribution of PSME-driven transcriptional activity will play a dominant role. Interestingly, the inability of PSES–TSTA to function in AR-negative DU145 (Fig. 2C) and PC-3 cell lines (data not shown) reaffirms previous reports that the androgen-suppressible activity of PSME is likely mediated through AR by an indirect mechanism (14). Further investigation of the transcriptional regulation of PSME is needed to resolve the interesting androgen-suppressive and prostate cancer–selective activity of this enhancer element.

Figure 5. PSES–TSTA imaging vector mediated detection of castration-resistant bony prostate cancer metastasis. A, time frame: 4- to 5-week-old male SCID-Beige mice were castrated. A total of $1 \times 10^5$ LAPC-4 AI tumor cells were injected into the right tibiae. Forty-two days later, the animals received $1 \times 10^5$ PFU of Ad-CMV-GFP (to blunt the vector uptake by Kupffer cells), followed by $4 \times 10^5$ PFU Ad-PSES-TSTA-FL through tail vein 4 hours later. FL BLI, $^{18}$F-NaF, and $^{18}$F-FDG PET-CT imaging was done at indicated times. B, Ad-PSES-TSTA directed FL BLI. Mice are displayed according to radiance intensity emitted from the right tibiae. The 2 mice on the left were negative whereas the other 7 mice were positive for tumor. Note the difference in intensity scales for mice 1 to 7 and mice 8, 9. C, immunohistochemistry staining of the right tibiae of 6 representative animals using a human-specific pan-cytokeratin antibody. D, $^{18}$F-NaF and $^{18}$F-FDG PET signals from tumor-bearing mice. The quantification represents % injection dose/g from left (control) and right (tumor injected) tibial ROI. Bar, 500 μm.
The advanced lethal stage of prostate cancer has a particular propensity to metastasize to bone, severely impacting the quality of life of the patient (27, 28, 38). The affinity for bone is likely due to protumorigenic growth factors and a favorable environment, produced as a result of the reciprocal interactions between prostate cancer and bone cells (39). ADT can induce factors that promote the process of prostate cancer bone metastasis (40). The current established clinical standard for imaging bone metastasis is whole-body planar bone scan with $^{99m}$Tc-methylenediphosphonate (MDP). Due to the low spatial resolution of bone scans, there is a favorable trend toward using $^{18}$F-NaF PET/CT with the hope of improving the sensitivity and resolution of bone metastasis detection (41). Several drawbacks of these 2 imaging modalities result from the fact that uptake of $^{99m}$Tc-MDP and $^{18}$F-NaF are reliant on bone remodeling, a later event in the bone metastatic process (42). For instance, both $^{99m}$Tc-MDP bone scan and $^{18}$F-NaF PET/CT will be unable to directly assess tumor volume, especially in the early stage of metastasis, or to concomitantly visceral metastatic lesions. These reasons could explain why specific detection of prostate cancer metastases in the tibial bone marrow was feasible via intravenous Ad-PSES-TSTA-FL injection/BLI, but not with $^{18}$F-NaF PET/CT. These data suggest that our model represents an early stage of CRPC osseous metastasis because we were unable to detect any bony deformity by inspection (Supplementary Fig. S3) or CT scan. In addition, the animals did not exhibit any abnormality in ambulation. The histologic analyses of the metastatic lesions also revealed the involvement of cancer cells in the bone marrow, but not the cortex of the bone. The clinical experience with FDG PET in detecting prostate cancer is equivocal (43, 44). Our experience indicates that human prostate cancer xenografts are not particularly avid in uptake of $^{18}$F-FDG (data not shown). This study further showed this point in bone marrow metastases of CRPC LAPC-4 tumor.

Several challenges are anticipated in the clinical translation of adenoviral-based reporter gene imaging described here. Significant hurdles to overcome will include the immunogenicity of the adenoviral vector. In particular, the preexisting immunity in a large proportion of the human population against the Ad5 serotype used here will likely induce rapid clearance of the vector. In addition, interaction of virus with blood-borne factors in the circulation could result in liver sequestration and thus hinder its biodistribution in human patients (45, 46). Recent advances shed light on promising strategies to overcome these challenges. For instance, on the basis of the recent reported ultrahigh resolution of viral capsid structure (47), one could mutate the virion capsule residues to ablate immunogenic epitopes, disrupt the interactions with circulating factors, or insert targeting ligands. The use of bispecific molecule has been successful in not only detargeting the native tropism of adenovirus in vivo but also retargeting the vector to tumor cell surface antigens (48). We and others have obtained promising results by applying advanced polymer technologies to coat the virus to blunt its immunogenicity as well as to enhance cell-targeted entry (49). The use of transient immunosuppression regimen has established traction in overcoming the immunogenicity issues of Ad gene therapy to facilitate long-term administration (50). In a very recent study, Bhang and colleagues showed that a nonviral cationic polymer vector can achieve efficient gene delivery and imaging to detect systemic metastasis in melanoma and breast cancer models (32). Their results also reaffirm the great specificity of transcription-based molecular imaging.

In summary, we report here the development of PSES–TSTA as an androgen-independent and prostate cancer–specific gene expression system. The transcriptional amplification strategy undertaken boosted the expression of both a bioluminescent reporter gene and a PET reporter gene, to achieve sensitive and prostate cancer–specific molecular imaging in preclinical models of CRPC. The potency, cell selectivity, and diagnostic potential of PSES–TSTA vectors was illustrated by the ability of a PSES–TSTA imaging vector to detect early CRPC bone marrow metastases following systemic vector administration. The PSES–TSTA vectors can be modified to simultaneously express imaging reporter genes and cytotoxic therapeutic genes. Hence, the implementation of multipronged therapeutic strategy, such as combined ADT with image-guided gene therapy, to treat advanced and even metastatic CRPC may be feasible using the PSES–TSTA system described in this study.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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