Breast cancer is the second leading cause of cancer-related death in women in the United States, accounting for an estimated 26% of all new cancer cases (1). In 2008 alone it was estimated that approximately 180,000 women were diagnosed with breast cancer, while over 40,000 died because of this disease. As is the case with most cancers, metastasis is the primary cause of mortality associated with breast cancer. Data assessing the 5-year relative survival rates of women diagnosed with breast cancers indicate a 6-fold increase in survival in women diagnosed with early-stage breast cancers, compared with those with advanced-stage breast tumors (2). Although detection and staging modalities of breast cancers have advanced significantly over the last decade, further effort is needed to ensure that mortality associated with this disease is decreased.

The spread of cancer cells to regional lymph nodes occurs through the lymphatic vasculature and is the first step in the dissemination of breast cancers (3). Hence, lymph node metastasis is a significant prognostic indicator in the course of this disease and often dictates therapeutic aggressiveness. A commonly used method to assess nodal status is the sentinel lymph node biopsy, which is based on the removal and histopathologic examination of the first draining, or sentinel lymph node, as determined by lymphatic uptake of radio-labeled colloids or dyes. In sentinel lymph node biopsy, the administration of lymphotropic dyes or radiolabeled colloids will drain through tumor-associated lymphatic vessels, leading to the localization of sentinel lymph nodes by visual inspection or through the use of a \( \gamma \) camera in the case of dyes or 99m technetium labeled colloids, respectively (4). However, sentinel lymph node biopsy is invasive and can lead to complications, such as shoulder dysfunction, nerve damage, upper arm numbness, and lymphedema (5). Thus, there is a great need for highly sensitive, accurate, and yet noninvasive detection modalities. Of interest, recent reports by our group and others illustrated through careful in vivo biodistribution studies that human serotype 5 adenoviral vectors (Ad) can traffic effectively into regional lymph nodes (6). In much the same way as the other lymphotropic agents, the uptake of adenovirus is influenced by its size (~100 nm) and charge (6, 7). Moreover, its lymphotropic properties can be exploited to detect nodal metastases by incorporating imaging reporter genes into the adenoviral vector (7, 8).

Systemic targeting of metastatic tumors cells is the ultimate goal in the field of cancer gene therapy and can potentially play...
**Translational Relevance**

This study illustrates the proof-of-principle of a novel technology to detect metastatic breast cancers. By engineering a recombinant adenoviral vector to produce imaging signal exclusively in a cancer-selective manner, our aim is to create a vehicle that can seek out and achieve functional molecular imaging of metastatic lesions in living subjects. In this study we combined optical imaging with clinically relevant positron emission tomography to specifically identify lymph node and liver metastases. Compared with conventional staging methods that require surgical biopsy of draining lymph nodes, the noninvasive and specific nature of our functional imaging enables the direct mapping of nodal involvement, and hence the prospect of reduced complications of this procedure. Another promising advantage of our gene expression – based strategy is that we can easily integrate the simultaneous expression of cytotoxic genes with the imaging gene into a single adenoviral vector. In doing so, we can achieve image-guided therapy to treat metastatic disease for breast cancer, as well as other types of cancer.

**Materials and Methods**

**Adenoviral generation.** Adenoviral vectors were constructed based on the AdEasy system (21). Virus was propagated, purified, and titred as previously described (9). The tail-to-tail configuration of the AdMUC1-TSTA-fl was constructed by replacing the PSEBC promoter from pShuttleBC-VF2 with a multiple cloning site. The MUC1 promoter, kindly provided by Dr. Noriyuki Kasahara, was subcloned from pShuttleBC-VP2 with EcoRI and EcoRV sites in the multiple cloning site, resulting in pShuttleMUC1-VF2. G5-fluc, obtained from pG5-fluc (22), was then inserted into pShuttleMUC1-VF2 in order to generate pShuttleMUC1-TSTA-fl.

**Cell lines and cell culture experiments.** The HepG2 liver tumor cell line (a kind gift from Dr. Edward McCabe), and the breast cancer cell lines KPL-1 (a kind gift from Dr. Dennis Slamon), MDA-MB-231, MDA-MB-435 (a kind gift from Dr. Luisa Iruela-Arispe), MCF-7, and BT-20 (obtained from the American Type Culture Collection) were all maintained in DMEM containing 10% fetal bovine serum and 1% penicillin/streptomycin. T47D breast cancer cells (obtained from the American Type Culture Collection) were cultured in RPMI containing 10% fetal bovine serum and 1% penicillin/streptomycin.

For transfection experiments, 0.5 × 10^6 cells were plated in 24-well plates and cotransfected in triplicate with equimolar amounts of either pGL3MUC1 or pMUC1-TSTA-fl and a CMV-β-galactosidase (β-gal) plasmid by standard LipofectAMINE 2000 protocol (Invitrogen). For adenovirus infection experiments, cells were plated at 0.5 × 10^5 in 24-well plates 24 h prior to infection, and infected with either AdCMV-fl or AdMUC1-TSTA-fl at a multiplicity of infection of 5 (MOI = 5). Forty-eight hours after transfection or transduction, cells were lysed and assayed by standard luciferase assay (Promega). β-Gal expression assessed by β-gal detection kit II (Clontech) was used to normalize for transfection efficiency.

**Tumor xenograft experiments.** Marked tumor models were generated by transduction of tumor cells with lentivirus carrying the cytomegalovirus (CMV) promoter-driven renilla luciferase (RL) reporter genes, as previously described (23). To promote lymphatic metastasis, tumor cells were transduced at a multiplicity of infection of 1 (MOI = 1, 1 infectious virus per cell) with a second lentiviral vector, pCCL-CMV-VEG-C-ires-EF (VFC) or empty vector pCCL-CMV-ires-EF to serve as control (Ctrl). KPL-1/RL tumor cells (5 × 10^5) were implanted with matrigel into the second mammary fat pad of female SCID/Beige mice. Tumor kinetics were monitored over 4 wk by bioluminescent imaging of renilla luciferase.

**In vivo off-target expression analysis.** Female Scid/Beige mice were i.v. injected with either AdCMV-fl or AdMUC1-TSTA-fl (1 × 10^4 pfu/100 μL). The animals were optically imaged for firefly luciferase activity at 5, 10, and 20 d postinfection. Briefly, mice were injected with D-luciferin (150 mg/kg i.p.) and imaged with the bioluminescent imager (IVIS 100; Caliper Life Science Inc.) after a 20 min incubation. At 20 d postinjection the animals were sacrificed and the indicated organs were extracted and imaged ex vivo. Data were analyzed using IGOR-PRO Living Image software as described previously (23).

**Targeting lymph node metastasis.** Mice received peritumoral injections of AdMUC1-TSTA-fl (1 × 10^6 pfu) 4 wk after mammary pad implantation of KPL-1/VEG-C cells. Three days after adenovirus injection, the axillary lymph nodes of treated animals were imaged for renilla and firefly luciferase expression, as described previously (8). Briefly, ipsilateral and contralateral lymph nodes were dissected and imaged by successive exposure to D-luciferin and coelenterazine. To minimize signal carryover in this dual imaging technique, the animals first received i.p. injections of D-luciferin. Twenty minutes after substrate administration, the animals were sacrificed, and the lymph metastasis of breast cancer. We believe that this system holds promise to be a useful tool for the clinical management of advanced-stage breast cancer.
PET/CT imaging. The mice were imaged by positron emission tomography (PET) using 200 μCi of $2^{[18]}$F-fluoro-2-deoxy-D-glucose ($^{18}$FDG) in a single i.v. bolus and imaged in a Focus 220 micro-PET scanner and MicroCAT II scanner (Siemens Preclinical Solutions) as previously described (8). PET images were created by filtered back projection at 0.4-mm pixel size with 0.8-mm slice thickness and fused PET-computed tomography (CT) images as previously indicated (24).

Statistical analysis. Characterization of MUC-1 constructs in cell culture experiments was carried out in triplicate, and data are presented as means ± SE. For analysis of VEGF-C–induced lymph node metastasis, data are presented as means ± SE and compared by Student’s t test. The correlation plot assessing targeting of lymph node metastasis was also analyzed using Student’s t test.

Results

The development and evaluation of the mucin1 TSTA system. In order to assess the efficiency and specificity of the Mucin1 promoter within the TSTA system, the activity of our pMUC1-TSTA-fl plasmid construct was first compared with a conventional one-step MUC1 direct expression vector (pGL3MUC1) in vitro (Fig. 1B). Both pMUC1-TSTA-fl and pGL3MUC1 constructs were transfected into both breast (MCF-7, BT-20, MDA-MB-231) and nonbreast cancer cell lines (HepG2), and firefly reporter activity was assayed. The selected cell lines were chosen based on the variability of their MUC1 expression as assessed in previously published results (15), and confirmed through reverse transcription-PCR analysis (data not shown). In MCF-7 cells, normalized firefly luciferase expression in one-step direct expression construct was observed to be 1,200 ± 90 RLU/s, and was amplified to 2.23 ± 0.22 × 10⁵ RLU/s in the MUC1 TSTA construct. In BT-20 cells, normalized one-step firefly luciferase expression was observed to be 850 ± 100 RLU/s, and 2.18 ± 0.15 × 10⁵ RLU/s in MUC-1 TSTA transfected cells. Based on these results, we determined that through the use of TSTA, we could amplify MUC-1 promoter expression approximately 180- and 260-fold in MCF-7 and BT-20 breast cancer cells, respectively. The expression profiles between pMUC1-TSTA-fl and pGL3MUC1 in all breast and nonbreast cancer cell lines were nearly identical, indicating that amplification occurs without a loss of promoter specificity. In addition, reporter activity directly correlated with previously established MUC1 expression levels in each cell line, giving further evidence of specificity.

We next integrated the Mucin-1 TSTA construct into an adenoviral vector, generating AdMUC1-TSTA-fl. We then compared the expression levels of this virus with those obtained by a CMV-driven firefly luciferase–expressing adenovirus (AdCMV-fl) in breast cancer (BT20, MCF-7, KPL-1, T47D, MDA-MB-231, and MDA-MB-435) and nonbreast (HepG2) cell lines, as previously described (8). PET images were created by filtered back projection at 0.4-mm pixel size with 0.8-mm slice thickness and fused PET-computed tomography (CT) images as previously indicated (24).

Targeting hepatic implants. Tumors were allowed to grow over a period of 1 mo, after which time the animals received i.v. injections of AdMUC1-TSTA-fl at $1 × 10^7$ pfu/mouse. The animals were optically imaged for firefly luciferase expression in each animal once a week for a period of 3 wk. Following imaging, the animals were sacrificed and their livers were harvested. Half of each of the four lobes of the liver were fixed in 3% paraformaldehyde, embedded in paraffin, sectioned, and stained with H&E, as described previously (23). The remaining liver lobes were microdissected and homogenized, and 10 mg of homogenate were lysed in passive lysis buffer. Five microliters of supernatant were assayed using dual luciferase assay (Promega).

PET/CT imaging. The mice were imaged by positron emission tomography (PET) using 200 μCi of $2^{[18]}$F-fluoro-2-deoxy-D-glucose ($^{18}$FDG) in a single i.v. bolus and imaged in a Focus 220 micro-PET scanner and MicroCAT II scanner (Siemens Preclinical Solutions) as previously described (8). PET images were created by filtered back projection at 0.4-mm pixel size with 0.8-mm slice thickness and fused PET-computed tomography (CT) images as previously indicated (24).

Statistical analysis. Characterization of MUC-1 constructs in cell culture experiments was carried out in triplicate, and data are presented as means ± SE. For analysis of VEGF-C–induced lymph node metastasis, data are presented as means ± SE and compared by Student’s t test. The correlation plot assessing targeting of lymph node metastasis was also analyzed using Student’s t test.

Results

The development and evaluation of the mucin1 TSTA system. In order to assess the efficiency and specificity of the Mucin1 promoter within the TSTA system, the activity of our pMUC1-TSTA-fl plasmid construct was first compared with a conventional one-step MUC1 direct expression vector (pGL3MUC1) in vitro (Fig. 1B). Both pMUC1-TSTA-fl and pGL3MUC1 constructs were transfected into both breast (MCF-7, BT-20, MDA-MB-231) and nonbreast cancer cell lines (HepG2), and firefly reporter activity was assayed. The selected cell lines were chosen based on the variability of their MUC1 expression as assessed in previously published results (15), and confirmed through reverse transcription-PCR analysis (data not shown). In MCF-7 cells, normalized firefly luciferase expression in one-step direct expression construct was observed to be 1,200 ± 90 RLU/s, and was amplified to 2.23 ± 0.22 × 10⁵ RLU/s in the MUC1 TSTA construct. In BT-20 cells, normalized one-step firefly luciferase expression was observed to be 850 ± 100 RLU/s, and 2.18 ± 0.15 × 10⁵ RLU/s in MUC-1 TSTA transfected cells. Based on these results, we determined that through the use of TSTA, we could amplify MUC-1 promoter expression approximately 180- and 260-fold in MCF-7 and BT-20 breast cancer cells, respectively. The expression profiles between pMUC1-TSTA-fl and pGL3MUC1 in all breast and nonbreast cancer cell lines were nearly identical, indicating that amplification occurs without a loss of promoter specificity. In addition, reporter activity directly correlated with previously established MUC1 expression levels in each cell line, giving further evidence of specificity.

We next integrated the Mucin-1 TSTA construct into an adenoviral vector, generating AdMUC1-TSTA-fl. We then compared the expression levels of this virus with those obtained by a CMV-driven firefly luciferase–expressing adenovirus (AdCMV-fl) in breast cancer (BT20, MCF-7, KPL-1, T47D, MDA-MB-231, and MDA-MB-435) and nonbreast (HepG2) cell lines, transducing at a multiplicity of infection of 5 (MOI = 5, 5 infectious virus per cell). AdMUC1-TSTA-fl consistently achieved higher reporter activity than AdCMV-fl in all breast cancer cell lines evaluated (Fig. 1C). The greatest difference in activity was observed in MCF-7 cells, in which firefly luciferase activity was observed to be 1.08 ± 0.07 × 10⁷ RLU/s in AdMUC1-TSTA-fl–infected cells compared with 6.72 ± 1.04 × 10⁵ RLU/s in the AdCMV-fl–infected cells. In KPL-1 cells, the respective AdMUC1-TSTA-fl and AdCMV-fl activity was 1.25 ± 0.02 × 10⁷ and 4.74 ± 0.85 × 10⁶ RLU/s.

The off-target expression of AdMUC1-TSTA-fl. It has been well established that applications of adenoviral vectors exhibit a
strong tropism for the liver in vivo (6). To evaluate the off-target expression of AdMUC1-TSTA-fl in the liver, we compared its activity with that of the strong constitutive AdCMV-fl (Fig. 2). Following i.v. injection with $1 \times 10^7$ pfu of each virus, the animals were monitored longitudinally for firefly luciferase expression by bioluminescence imaging. High liver transduction and expression was observed with the AdCMV-fl–injected group ($n = 6$; Fig. 2A). However, due to the tissue selectivity of the MUC-1 promoter, the AdMUC1-TSTA-fl–injected animals ($n = 6$) exhibited no detectable signal in liver despite the majority of virus trafficking to and transducing this organ (Fig. 2A). The magnitudes of luciferase expression in the major organs of the animals in each treatment group were determined at the day 20 end point by ex vivo bioluminescent imaging (Fig. 2B).

**KPL-1 xenograft model of breast cancer.** We next set out to evaluate the specificity of AdMUC1-TSTA-fl in xenograft models of breast cancer. KPL-1 was recently established as a novel human breast cancer cell line derived from the malignant effusion of a 51-year-old Japanese breast cancer patient (25). Previously published reports (15) and our experience has shown that these cells express high levels of mucin1 RNA (data not shown). In addition, this model has been shown to exhibit a high tumor take rate in vivo. As a result, we elected to use KPL-1 breast tumors for implantation into the mammary fat pads of female SCID-beige mice, in order to evaluate the targeting ability of AdMUC1-TSTA-fl. Robust firefly luciferase signal was detected in the primary tumor after intratumoral injection of $1 \times 10^8$ pfu of AdMUC1-TSTA-fl (Fig. 3A). Although a large percentage of the virus accumulates in the primary tumor, our prior experience indicates that systemic leakage of viral vector is common after tumor-directed injection leading to signal in distant sites such as the liver (11, 26). This is due in large part to the high affinity of the virus for this organ. The lack of systemic signal outside of the tumor (Fig. 3A) again reaffirms the tumor-selective expression of AdMUC1-TSTA-fl.

**Inducing lymph node metastasis in KPL-1 tumors by VEGF-C overexpression.** Based on our experience, the orthotopic KPL-1 xenografts, established in mammary fat pads of immune deficient mice, displayed a low incidence of nodal metastasis following implantation of $5 \times 10^5$ cells. To augment the rate of nodal metastasis, we induced VEGF-C overexpression in the KPL-1 tumors. Extensive literature supports that enhanced tumoral lymphangiogenesis promotes tumor cell dissemination via lymphatic vasculature to draining lymph nodes (3, 27). The dominant lymphatic growth factors are VEGF-C and VEGF-D, which induce lymphatic endothelial cell proliferation and migration through the activation of the receptor, VEGFR-3 (3). Experimental models of breast cancer have shown that VEGF-C expression enhances lymphangiogenesis and subsequent lymph node metastasis (27). In this study, we achieved stable expression of VEGF-C in KPL-1 cells through the use of a lentiviral vector expressing VEGF-C and green fluorescent protein, driven by the CMV promoter (ref. 23; Fig. 3C). The control KPL-1 tumor, lacking VEGF-C overexpression (non VEGF-C, Fig. 3B), was transduced with the lentiviral vector that only expresses green fluorescent protein. To facilitate the tracking of tumor growth and metastasis, all cells were marked with a second lentiviral vector expressing renilla luciferase. Following tumor implantation, animals ($n = 16$) were imaged for renilla luciferase over the course of four weeks (Fig. 3B and C). At the terminal time point, the animals were sacrificed and axillary lymph nodes were extracted and assessed for nodal involvement by renilla luciferase optical signal. Imaging revealed that 0 of 8 non-VEGF-C control tumor-bearing mice exhibited lymph node metastasis (Fig. 3D), whereas 4 of 8 mice bearing VEGF-C–overexpressing KPL-1 tumors had detectable lymph node metastasis ($P < 0.05$; Fig. 3E). A region of interest was taken for each axillary node and the average of optical signal within the region of interest for positive lymph nodes was then compared with the average of all negative nodes (Fig. 3F).

**Targeting lymph node metastasis.** Next, we tested the ability of the AdMUC1-TSTA-fl to target and detect lymph node metastasis in the above described renilla luciferase–marked KPL-1-VEGF-C orthotopic breast tumors. Targeting experiments were carried out using peritumoral injections of $1 \times 10^8$ pfu of AdMUC1-TSTA-fl after the tumors were grown.

---

**Fig. 2.** In vivo off-target expression analysis. Non–tumor-bearing female SCID mice were given i.v. injections of either AdCMV-fl ($n = 8$) or AdMUC1-TSTA-fl ($n = 6$) virus at $1 \times 10^7$ pfu. A, mice were optically imaged for firefly luciferase expression at 5, 10, and 20 d postinjection. Three representative animals from each cohort were shown. B, at day 20, the mice were sacrificed, and their organs (liver, lung, spleen, kidney) were harvested and imaged for firefly luciferase expression ex vivo (photons/s/cm²/sr²).
for approximately four weeks in female SCID beige mice \((n = 8)\); Fig. 4A). Three days post–AdMUC1-TSTA-fl injection, the animals were sacrificed and both the ipsilateral and contralateral axillary lymph nodes relative to the primary tumor were extracted and assessed for renilla and firefly luciferase expression by sequential \textit{ex vivo} optical imaging (Fig. 4B). Strong optical signals were observed in the ipsilateral lymph nodes of 50% of the treated cohort, including the representative animal (Fig. 4B), showing correlation between the vector-directed firefly luciferase signal and the renilla luciferase tumor signal. To obtain a more precise measurement of these results, we opted to carry out an \textit{in vitro} analysis of both firefly and renilla luciferase expression in the extracted lymph nodes using a dual luciferase assay. Through this analysis we were able to further correlate the presence of lymph node metastasis (renilla luciferase expression) with viral reporter gene expression (firefly luciferase expression). Renilla expression was plotted against firefly luciferase expression giving an \(R^2\) value of 0.689 \((P < 0.05);\) Fig. 4C). These findings support that the AdMUC1-TSTA-fl we have created is able to target established MUC1-positive KPL-1 nodal metastasis.

**Targeting experimental hepatic metastases.** To further scrutinize the tumor selectivity of the MUC-1–targeted vector, we chose to target liver metastasis by systemic vector administration. This is a clinically relevant scenario, as breast cancer patients with advanced disease will often present with secondary tumors in the liver, lung, and bone (28). For this set of experiments, \(5 \times 10^5\) KPL-1 cells were surgically implanted into the livers (left lobe) of four-week-old SCID-beige mice \((n = 5)\) and \(1 \times 10^5\) pfu of AdMUC1-TSTA-fl was injected i.v. three weeks after tumor cell implantation. Repetitive imaging for firefly luciferase activity revealed localized signals over the hepatic area, which persisted over the three-week duration of monitoring (Fig. 5A). To achieve more precise three-dimensional localization of the hepatic tumor in the animal, a combined PET-CT modality with 18FDG as the tracer was used. PET imaging revealed a predominant FDG-avid signal in the hepatic region (Fig. 5A). The vector-directed luciferase signal also initiated more prominently in the hepatic region. However, the known tissue-scattering effects of optical signals make their precise localization difficult. However, side by side comparison of virus-injected, non–tumor-bearing control animals suggests that we are able to achieve tumor-specific reporter expression. Interestingly, in the immune deficient setting, the vector-directed luciferase expression was able to persist and increase incrementally over time. Histologic examination of the liver tumor at the end point of the study (35 days after implantation) revealed large well-demarcated tumors (measuring \(~1\) cm) that had invaded extensively into the left lobe of the liver (Fig. 5B). Comparison of optical signal between animals bearing KPL-1 hepatic implanted tumors and control non–tumor-bearing mice \((n = 3);\) Fig. 5A), both injected i.v. with the same titer of AdMUC1-TSTA-fl, supports our belief that viral gene expression comes as the result of tumor targeting. Importantly, despite the high amount of virus that is sequestered into liver cells as a
result of the viral liver tropism, the strong specificity and robust gene expression of our virus allowed for targeted imaging of these KPL-1 lesions alone. To verify the ability of AdMUC1-TSTA-fl to target the hepatic KPL tumors, the harvested liver of a representative animal (Fig. 5C) was divided by lobe, and assessed for both firefly and renilla luciferase expression. The dual luciferase activity measurements from the distinct lobes again confirmed the correlation between tumor presence and viral gene expression. These results showed the ability of AdMUC1-TSTA-fl to specifically target breast tumors in the liver via i.v. injection.

Discussion

The development of novel targeted tools for use in the early detection and treatment of metastatic breast cancer is critical for reducing mortality associated with this disease. In this current study, a transcriptionally targeted, recombinant adenovirus served as a diagnostic tool by delivering imaging reporter genes to tumor cells, enabling the direct detection and visualization of tumor metastasis in lymph nodes and liver. This system made use of the tumor-specific MUC1 promoter in conjunction with the TSTA system, a two-tiered amplification scheme that has been applied successfully to modify the prostate-specific antigen promoter (10, 22), the VEGF promoter (29), pancreatic-specific cholecystokinin type A receptor promoter (30), and the survivin promoter (31). Through adaptation of TSTA, mucin-1 promoter activity was amplified up to 260-fold, greatly enhancing its sensitivity and efficiency.

Systemic application of Ad vectors has been limited by their strong tropism for the liver, which diminishes gene delivery to other organs (6). Over the years much work has focused on abolishing liver transduction of adenovirus through various strategies. Some include modifications to the adenoviral structure (32, 33), retargeting the virus through use of adapters (34), and most recently through ablation of FX-facilitated transduction (35, 36). However, strategies to detarget the adenovirus away from the liver have not shown enhanced transduction to extrahepatic target sites. We and others have shown that transcriptionally targeted adenoviral vectors could be a means to safeguard against unwanted liver transduction (11, 37). Although a transcriptional targeting strategy will not prevent adenoviral liver infection, it can eliminate viral gene expression in the liver. In this study, we showed that the mucin-1 promoter–driven AdMUC1-TSTA-fl remained transcriptionally silent in the liver due to weak mucin-1 expression.
in normal liver tissue. Conversely, this cancer-selective imaging Ad can successfully target and produce optical signals to detect breast tumor lesions in the liver following i.v. administration. To localize the metastatic lesions more precisely, we did PET-CT scans that enabled us to visualize the heightened glucose metabolism of tumors by the FDG tracer. By combining two molecular imaging techniques that provide metabolic and gene expression information, conferred by FDG-PET and bioluminescence imaging respectively, the specificity of detecting metastases can be greatly enhanced.

Besides strong liver tropism, human adenoviruses exhibit preferential distribution into the lymphatic system (7, 8). Its lymphotropic properties can be gleaned from historical evidence that the virus was isolated from adenoids, which collect the majority of lymph drainage of upper respiratory tracts (38). In a recently published report, we were able to successfully target and express PET imaging reporter genes in metastatic prostate cancer tumor cells within draining lymph nodes with a prostate-specific TSTA Ad (8). This current study further verifies the feasibility of exploiting the inherent lymphotropic properties of Ad to query the sentinel lymph node status in a breast tumor model. Employing an approach that parallels clinical lymphoscintigraphy, the AdMUC1-TSTA-fl viral particles were injected peritumorally. We observed that the tumor-selective firefly luciferase signals correlated directly with the Renilla luciferase signals of the marked tumor cells in the sentinel lymph nodes of the breast tumor–bearing cohort. This finding indicates that AdMUC1-TSTA-fl is specific and efficient at detecting sentinel lymph node metastases. Compared with conventional lymphoscintigraphy and the sentinel lymph node biopsy method, this transcriptionally targeted adenoviral-based method of lymphangiography has the advantage of being a noninvasive imaging modality, obviating the need to harvest draining nodes for histologic analyses, thereby eliminating the risk of side effects associated with sentinel lymph node biopsy.

A recent insightful review by Punglia et al. (39) examined the findings of 78 randomized clinical trials in the Early Breast Cancer Trialists’ Collaborative Group evaluating the extent of surgery and the use of radiation therapy (40). The definitive
message from this thorough review is that improved local control, such as excision to negative margins and the use of additional radiation doses, provided a highly statistically significant improvement in long-term breast cancer survival. The most significant indicators for disease recurrence and poor outcome as reported in this study and others include the number of lymph nodes involved, tumor size and grade, lymphovascular invasion, mitotic index, and Her2 positivity (39, 41, 42). According to these perspectives, the ability of AdMUC1-TSTA-fl to target lymph node metastasis might provide an additional tool to achieve better local control. Not only can the tumor-selective Ad be used to express various imaging genes for tumor staging, but the same strategy can be applied to express cytotoxic genes to eradicate lymph node metastasis, offering the potential to reduce systemic tumor spread. The TSTA expression system is particularly amenable to simultaneously express multiple transgenes (43). In particular, we have succeeded in expressing an imaging gene and a therapeutic gene simultaneously using the survivin tumor-specific promoter (31), the MUC1 promoter (44), and the prostate-specific antigen promoter.7

We anticipate that a significant challenge to the delivery of gene expression–targeted interventions to the clinic, such as the one described here, would be the immunogenicity of the adenoviral vector (45). To overcome this limitation, and we and others have undertaken several approaches to modify the surface properties of the viral capsid in order to reduce its antigenicity and to enhance its stability in circulation (37, 46). Other steps taken include transiently dampening the host immune system to prolong vector gene expression (47). Recently, we have adapted a method to synthesize block copolymers with precise control of chain lengths and compositions (48). In turn, these synthetic polymers are used to coat the adenovirus by noncovalent electrostatic interactions. Coated adenoviruses have shown significant improvement in their transductional efficiency in cell culture and in animals.8 This polymer coating approach in conjunction with further transductional targeting modifications (34) could greatly enhance the specificity and performance of our MUC1 expression–targeted Ad in living subjects. Combination use of this system with various transductional targeting strategies would be a vast improvement upon currently available technologies.

In this study, we illustrated in principle the ability of the Ad-based TSTA gene transfer technology to deliver and express imaging reporter genes specifically in breast tumor metastases in living subjects. Due to the low energy of visible light, optical imaging approaches, such as bioluminescence imaging employed in this study, are unable to detect signals beyond a few centimeters of depth as the photons are scattered or absorbed by tissue. We envision the use of PET-CT to be much more appropriate in the clinics and we recently reported the feasibility of employing a parallel Ad-mediated functional PET imaging method to detect prostate cancer nodal metastasis (8). To continue refining this technology for extension into human studies, we are focused on expanding its targeting capabilities with additional promoters, combining with transductional modifications to increase specificity, and integrating clinically relevant PET-CT approaches. We believe that this system holds great promise and has the potential to significantly impact diagnostic and treatment options for breast cancer patients.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We deeply appreciate the technical support provided by Dr. Marxa Figueiredo, Dr. Mai Johnson, Yiwei Lin, and Oh-Joon Kwon.

7 Sato et al, unpublished data.

8 Koh et al, unpublished data.

**References**


20. Block A, Milasimovic D, Mueller J, Schaefer P, Schaefer H, Goren H. Amplified MUC1-specific gene expression in colon cancer cells utilizing a binary...
A Potent, Imaging Adenoviral Vector Driven by the Cancer-selective Mucin-1 Promoter That Targets Breast Cancer Metastasis

Steven T. Huyn, Jeremy B. Burton, Makoto Sato, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/15/9/3126

Cited articles
This article cites 46 articles, 10 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/9/3126.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/15/9/3126.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.