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Molecular Imaging of Reporter Gene Expression in Prostate Cancer: An Overview

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Prostate cancer remains an important and growing health problem. Advances in imaging of prostate cancer may help to achieve earlier and more accurate diagnosis and treatment. We review the various strategies using reporter genes for molecular imaging of prostate cancer. These approaches are emerging as valuable tools for monitoring gene expression in laboratory animals and humans. Further development of more sensitive and selective reporters, combined with improvements in detection technology, will consolidate the position of reporter gene imaging as a versatile method for understanding of intracellular biological processes and the underlying molecular basis of prostate cancer, as well as potentially establishing a future role in the clinical management of patients afflicted with this disease.

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Nephrourological cancers, and especially prostate cancer, will remain an important and growing health problem for the foreseeable future. Prostate cancer is the most common nonskin cancer and the second leading cause of cancer deaths among men in most Western countries.[1] It has a worldwide incidence of 25.3 per 100,000, with large differences between countries.[2] The mortality rate of 8.1 per 100,000 mainly affects men at older ages. In the United States, approximately 230,000 new cases of prostate cancer were diagnosed in 2004.[3]

Recent improvements in prostate cancer detection have narrowed the gap between the incidence and the prevalence of prostate cancer. This, however, does raise some concerns about the risk of overdetection of latent cancers which, in turn, establishes a need for improvement in screening strategies to better identify clinically significant disease. Advances in imaging of prostate cancer may lead to early neoplasm detection and more accurate tumor staging, with consequent improved and adequate treatment, better monitoring of the disease, and enhanced surveillance for recurrences after treatment.[4]

Prostate Cancer: Overview of Current Conventional and Metabolic Imaging Techniques

There appears to be no consensus on the imaging of primary prostate cancer. Imaging of prostate cancer has conventionally relied on modalities such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), but with limited roles and success. Ultrasonography is mainly used for biopsy guidance and brachytherapy seed placement but, unfortunately, prostate cancer can be isoechoic and therefore indistinguishable from normal tissue on transrectal ultrasound.[5] The role of CT is essentially limited to assessment of advanced metastatic disease and although MRI is useful for local staging, there are issues with reproducibility of image quality and interobserver variability.[6,7]

Prostate cancer unfortunately shows a wide variation in biological behavior from a nonaggressive, silent, intraprostatic type to a very aggressive, bone-metastasizing type. This is compounded by the many other benign conditions affecting the prostate, which makes distinguishing prostate cancer even more of a challenge. More accurate disease characterization during the next decade is likely to arise from the development and implementation of more functional and molec-

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ular imaging approaches. Molecular imaging may be broadly defined as the visual representation, characterization, and quantification of biological processes at the cellular and subcellular levels within intact living organisms. In the assessment of prostate cancer, molecular imaging might offer the potential to: (1) understand abnormal pathology at a molecular level in a noninvasive manner; (2) diagnose and characterize disease much earlier; and (3) assess response to therapy with greater speed and accuracy. Recent general reviews on the subject of molecular imaging are available elsewhere, including accounts of the 5 general requirements (target selection, probe development, overcoming of biological barriers, signal amplification, and use of imaging instruments) for performing molecular imaging. We also recently reviewed the general principles that determine the ever-increasing integration of in vivo molecular imaging techniques into molecular medicine applications.[8-10]

A "direct" molecular imaging strategy (see below) using positron emission tomography (PET) with an ideal tracer would in theory be invaluable in distinguishing malignant from non malignant disease. At present however, there is no radiopharmaceutical which convincingly meets the desired criteria for prostate cancer imaging. For example, ^{18}F-fluorodeoxyglucose (^{18}F-FDG) imaging of prostate cancer has so far had very little success in detecting both primary and metastatic disease.[11,12] This is explained by the reduced uptake of FDG in prostate cancer cells attributable to a slower metabolic rate and reduced expression of glucose transport proteins. Additionally the renal excretion of FDG, with activity in the ureters and bladder, obscures visualization of the regions of interest. This is compounded by the spatial resolution of PET which only approximates 5 to 6 mm, and so limits its usefulness in evaluating local spread of prostate cancer, regional lymph node involvement and bone metastases.

A number of other radiopharmaceuticals have also been investigated for PET imaging, namely carbon-11-choline, carbon-11-acetate, fluorine-18-choline, fluorine-18-acetate, fluorine-18-testosterone, fluorine-18-fluoride, and carbon-11-methionine. The current evidence suggests that the radiopharmaceuticals available for PET imaging, including ^{18}F-FDG, at present offer no significant advantage over conventional imaging of primary prostate cancer. With ^{18}F-FDG, this is the result of poor uptake of FDG by cancer cells, lymph node, and bone metastases.[13] Despite its potential advantages, further prospective clinical trials are required to determine the role of PET in prostate cancer.[14-16] The hope is, that with further development of new tracers that are tailored to prostate cancer metabolism and the advent of the hybrid PET/CT modality offering supplemental anatomical information, PET imaging is likely to play a greater role in prostate cancer management.

Another molecular imaging modality capable of providing information on tissue microenvironments, magnetic resonance spectroscopic imaging (MRSI), is a noninvasive tool that combines magnetic resonance imaging (MRI) and spectroscopy to provide 3-dimensional anatomic and metabolic information. Prostate cancer in this instance can be evaluated beyond the morphologic information provided by MRI through the detection of cellular metabolites (eg, choline and citrate). An interesting recent study also looked at in vivo ^{19}F magnetic resonance spectroscopy and chemical shift imaging of tri-fluoro-nitroimidazole as a potential hypoxia reporter in a rat prostate tumor model.[17] There is a consensus that the supplementation of MRI with MRSI can improve cancer localization within the prostate and detect extracapsular spread and evaluation of cancer aggressiveness. A number of groups have reviewed the role of MRSI in prostate cancer imaging and described the benefits of MRSI in improving the sensitivity of MRI for localizing prostate tumor.[18,19] A recent study has evaluated the suitability of MRSI in screening for prostate cancer in comparison with T2-weighted MRI. It was shown that 3D-spectroscopy can improve the diagnostic accuracy of T2-weighted imaging alone.[20] Further prospective trials are however required to compare this novel technique to existing conventional imaging strategies to delineate the usefulness and role of MRSI in prostate cancer imaging.

Principles of Reporter Gene Expression Imaging

Alterations in normal regulation of gene expression result in disease processes, which may be caused by interactions with the environment, hereditary deficits, developmental errors, and the ageing process.[21,22] Previously only techniques such as tissue biopsy, blood sampling, histochemical, and immuno-histochemical staining could provide information on gene expression but without the ability to noninvasively determine the location, magnitude and extent of gene expression in a living subject.[23] As a subdiscipline of molecular imaging in living subjects, reporter gene expression imaging, involves the imaging of a gene product made via the 2 steps of transcription and translation. A reporter gene is one with a readily measurable phenotype that can be distinguished easily from a background of endogenous proteins.[24]

The principle behind reporter gene imaging involves the use of a pretargeting molecule, a reporter gene, which is introduced into the cells of living subjects and which may also be linked to a different gene under scrutiny (under the control of one of many possible promoters). The expression of the reporter gene then becomes linked to the activity of the gene which is being studied, for example, by sharing the same promoter/enhancer elements. The product of reporter gene expression (reporter protein) can then act as a "reporter" or 'signal' of the target gene activity by using a molecular probe (reporter probe) specific to the reporter protein which can be measured and imaged using various techniques. The "signal" may arise from a radioisotope, a photochemical reaction, or a magnetic resonance metal cation, depending on the nature of the reporter probe and the imaging instrumentation used.

Thus, a number of novel imaging methods can be used to localize, visualize, and quantify the signal, providing therefore the ability to image gene function. Reporter imaging systems can be categorized into 2 main groups: (1) the reporter protein is intracellular, for instance, thymidine kinase,

the luciferases, and green fluorescent protein (GFP) or (2) the reporter protein is associated with the cell membrane, for example, dopamine-2 receptor, somatostatin or transferrin receptor, or the sodium iodide symporter. The imaging modality used depends essentially on the reporter protein produced. More detailed reviews of PET-based (eg, herpes simplex virus type-1 thymidine kinase [HSV1-tk] enzyme with probes based on radiolabeled uracil nucleosides or acycloguanosine derivatives), single-photon emission computed tomography (SPECT)-based, MRI-based, fluorescent-based (eg, using green fluorescent protein), and optical bioluminescence-based (eg, using luciferases) reporter systems are available elsewhere.[21,25,26]

There are essentially 2 types of genes that can be targeted to image gene expression, genes externally transferred into cells of organ systems (transgenes) or endogenous genes. Most current reporter gene imaging applications use transgenes. Imaging an endogenous gene requires that a reporter gene be linked to the endogenous promoter, the switching on of which concurrently activates the reporter protein and, therefore, its detection indirectly heralds activation of the endogenous gene.[27,28]

The reporter gene expression strategy described previously in this review may be referred to as an indirect one. This is in contrast to a direct strategy that uses de novo synthesis of unique molecular probes targeted to a specific molecular target such as a receptor or enzyme. The relative merits of these approaches are discussed elsewhere.[21] The indirect strategy is versatile in that a single reporter probe/gene combination can be used with or without different endogenous promoters and genes to image a number of different biological processes. Application of reporter gene imaging, both in the laboratory and potentially in clinical practice, could provide the opportunity to learn much more about the pathogenesis of prostate cancer and potentially contribute to diagnosis and monitoring response to therapy, all at a subcellular and molecular/genetic level before phenotypic changes occur.

In this overview, focus has been placed on the use of bioluminescence reporter gene expression imaging for prostate cancer. This is a noninvasive optical imaging modality based on the enzymatic generation of visible light, allowing sensitive and quantitative detection of bioluminescence reporter genes in intact living small research animals. In recent years there has been huge growth and interest in its experimental use for molecular reporter gene imaging of cancer. More detailed descriptions and reviews of optical bioluminescence-based imaging can be found elsewhere.[21,26,29]

Experimental Applications of Reporter Gene Imaging in Prostate Cancer

There are 4 broad categories of experimental applications using reporter genes when imaging prostate cancer: (1) gene marking of cells with reporter genes; (2) imaging of gene therapies; (3) imaging of transgenic animals carrying reporter genes; and (4) imaging of more complex intracellular events such as protein-protein interactions and protein trafficking.

Imaging Gene-Marked Cells

Gene marking makes it possible to follow the behavior of cells in diverse locations in the body.[30] It is necessary to stably transfect cells with an imaging marker gene to allow these cells and their progeny to be followed for their entire lifespan within the living subject. In practice, however, if the gene marked cells are to be followed and imaged in the living subject for no more than 7 to 10 days, then transient transfection is sufficient depending on the cells in question and other parameters as well.[21] In general, there are 2 methods of gene-marking cells: (1) ex vivo transfection with a vector containing an imaging cassette followed by placement of the cells in a living subject; and (2) direct in vivo placement into the cells of interest within the living subject, usually by injection of the vectors carrying the reporter gene as part of recombinant genomes of viruses.

In clinical practice, gene marking has been applied mostly to hematopoietic cells.[30] In experimental applications in the study of prostate cancer, gene marking of cells that are static in one location can be used for (1) first assessment and continued validation of reporter genes and their probes, (2) refining the technical aspects of molecular imaging signal detection from the prostate, or (3) studying the behavior of gene-marked prostate cancer cells in living subjects, including the assessment of novel therapies to treat prostate cancer.[31]

There are a number of examples in the literature in which prostate cancer cells have been studied after gene marking with reporter genes, thus allowing temporal, spatial, and quantitative study of these cells. Jenkins and coworkers used the bioluminescent human prostate carcinoma cell line PC-3M-luc-C6 and a Firefly luciferase (Fluc)-based prostate cancer animal model to noninvasively monitor in vivo growth and response of tumors and metastasis before, during, and after chemotherapy.[32] Gene-marked bioluminescent PC-3M-luc-C6 cells, constitutively expressing Fluc, were implanted into the prostate or under the skin of mice for primary tumor assessment. Cells also were injected into the left ventricle of the heart as an experimental metastasis model. Serial in vivo whole-body images of anesthetized mice, either untreated or treated with 5-fluorouracil or mitomycin C, were then obtained to follow the gene-marked cells using optical bioluminescence imaging. Ex vivo imaging and/or histology was used to confirm the in vivo findings. The group was able to successfully detect and quantify early inhibition of subcutaneous and orthotopic prostate tumors in mice as well as significant tumor regrowth after treatment. It was also possible to image metastatic disease after the intracardiac injection of PC-3M-luc-C6 cells. Quantification of data by distinguishing differential drug responses and metastatic tumor relapse patterns over time depending on the metastatic site also was possible.

Iyer and coworkers developed a third-generation HIV-1-based lentivirus vector carrying a prostate-specific promoter

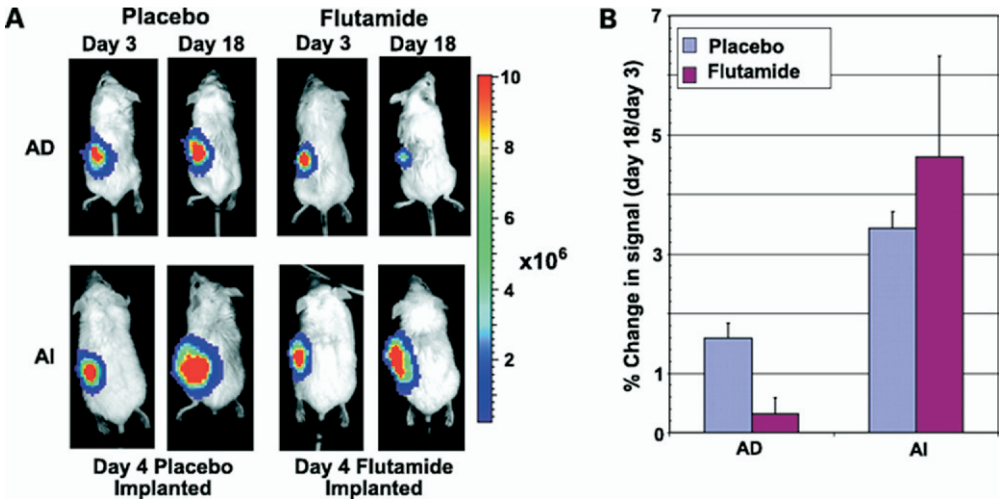

Figure 1 LAPC9 androgen-independent tumors are resistant to flutamide. AdTSTA were tail-vein injected into intact or castrated male mice bearing LAPC9 xenografts. (A) typical effects. On day 3, a baseline image was acquired and flutamide or placebo pellets were implanted. The effect of treatment at day 18 is shown. No flutamide effect was detected in the LAPC9 androgen-independent tumor model. (B) cohorts of androgen-dependent (n = 7) and androgen-independent (n = 6) animals were studied. Columns indicate percent change in signal versus day 3 in placebo- and flutamide-treated animals bearing androgen-dependent and androgen-independent tumors. (Reproduced with permission from Ilagan et al.[34])

to monitor the long-term, sustained expression of the *Fluc* reporter gene in living mice. *Fluc* in the transcriptionally targeted vector was driven by an enhanced prostate-specific antigen (PSA) promoter in a 2-step transcriptional amplification (TSTA) system. The system was used to evaluate lentivirus (LV-TSTA)-mediated gene delivery, cell-type specificity, and persistence of gene expression in cell culture and in living mice carrying prostate tumor xenografts.[33] Others have also used a TSTA system to, for example, test an androgen receptor (AR)-specific molecular imaging system in its ability to detect the action of the antiandrogen flutamide on AR function in xenograft models of prostate cancer (Fig. 1) and to assess whether the AR is fully functional in recurrent prostate cancer after androgen withdrawal.[34,35]

Another recent study described 2 new models that permit prostate imaging ex vivo, in vivo, and in utero, as well as the ability to use these models for detecting small metastasis and testing reagents that modulate the AR axis. Mice prostate epithelium was gene marked again with the *Fluc* using 3 composite promoters called human kallikrein 2 (hK2)-E3/P, PSA-E2/P, and ARR2PB, derived from hK2, PSA, and rat probasin regulatory elements, generating EZC1, EZC2, and EZC3-prostate mice, respectively. It was shown that these models allow noninvasive, longitudinal in vivo imaging of prostate tissue (Fig. 2), highlighting the usefulness for imaging prostate development, growth, metastasis, and response to treatment. Castration reduced *Fluc* expression by up to 97% in these models. Interestingly, the use of a gonadotropin-releasing hormone antagonist led to extensive inhibition of reporter activity, thus opening up the possibility for using these models of *Fluc*-based gene marking to develop and monitor improved drugs that could inhibit the AR axis.[36]

Molecular imaging of gene marked cells was used by El Hilali and coworkers in experimental studies to develop and validate novel imaging techniques potentially useful in prostate cancer.[37] This study used *Fluc*-expressing human prostate tumors and metastases in nude mice to compare the efficiency of noninvasive light detection with in vitro quantification of Fluc activity. An intensified charge coupled de-

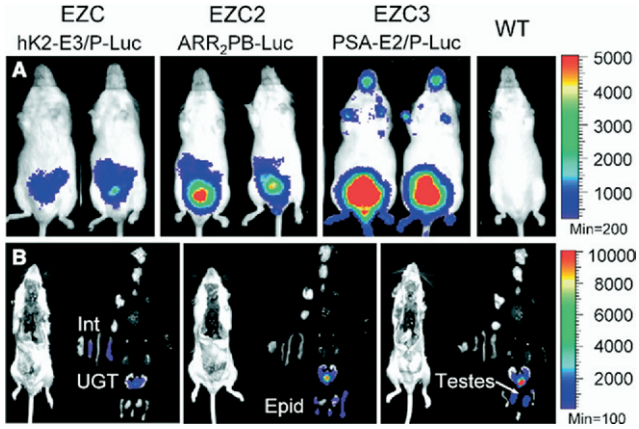

Figure 2 Comparison of three distinct prostate-specific promoters in transgenic mice. (A) Male 12-week-old transgenic mice were anesthetized and bioluminescence was imaged 10 minutes after intraperitoneal injection with D-luciferin (40 mg/kg). Two representative mice per line and one wild-type (*WT*) nontransgenic mouse are shown. (B) Mice were immediately euthanized and dissected. Isolated organs were laid out on nonluminescent paper in anatomically relevant order and imaged within 45 minutes. Most highly bioluminescent organs are labeled. Int, intestines; UGT, urogenital tract; Epid, epididymis. (Reproduced with permission from Seethammagari et al.[36])

vice (CCD) video camera was used to noninvasively image Fluc activity. The photon events recorded in tumor images were compared with the number of relative light units from luminometric quantification of homogenates from the same tumors. The study concluded that intensified CCD images alone were insufficient for quantitative evaluation of prostate tumor growth in mice. On the other hand, a combined videometric and luminometric approach did allow adequate quantification. As well as optical bioluminescence imaging, a dedicated small animal PET (microPET) scanner has also been successfully used to serially and noninvasively image prostate cancer in murine models, highlighting its potential clinical use in imaging of tumor metastases.[38]

One of the great advantages of gene marking cells is the ability to image cell trafficking in vivo. There are already examples in clinical practice, in particular in immunological and oncological studies, where cell labeling techniques are used to image cell trafficking in vivo eg, ^{111}In-Oxine for SPECT imaging of infection and inflammation. For in vivo imaging of cell trafficking, simple cell labeling has two distinct disadvantages which are overcome by gene marking. Firstly the imaging signal is not dependant on cell viability and may originate from extracellular spaces or from within immune scavenger cells, but when a gene marked cell dies, so does the imaging signal. Secondly, with gene marking the imaging gene is passed on to the cell progeny. Consequently the imaging signal is not lost through dilution or loss of the label from the cell.[39] A number of studies have therefore shown how gene marking can been used to study prostate cancer cell trafficking, and consequently provide a novel way to image prostate cancer metastases (Fig. 3).[32,36,40] For example, LeRoy and coworkers used a canine prostate carcinoma xenograft to evaluate and image neoplastic cell growth and osteoblastic metastases in nude mice using histoplathology, radiography, and bioluminescence imaging.[41] Interestingly, another group employed bioluminescence reporter gene imaging to investigate the role of tumor lymphangiogenesis during metastasis. Comparisons were made of the LAPC-4, LAPC-9, PC3, and CWR22Rv-1 cell lines from human prostate cancer xenograft models, labeled with the *Fluc* for optical imaging. After tumor implantation the animals were sequentially imaged for several months for the appearance of metastases. Metastatic lesions were confirmed by immunohistochemistry. Additionally, the angiogenic and lymphangiogenic profiles of the tumors were characterized.[42]

Imaging of Gene Therapies

Molecular imaging using reporter genes could play a vital role in optimizing gene therapy.[43,44] It is anticipated that, eventually, by the delivery of one or more transgenes to target tissue(s), it will be possible to successfully treat many diseases, including prostate cancer, as more is being understood about the genetic link with prostate cancer pathogenesis.[45,46] To date, however, gene therapy has had only limited success. A major challenge is to achieve controlled and effective delivery of genes to target cells as well as avoiding ectopic expression. Reporter gene imaging provides quantitative assessment of reporter gene expression and therefore the ability to provide inferred knowledge of extent, location, and duration of therapeutic gene expression. At present, there are a number of molecular biology strategies to link expression of a therapeutic transgene and an imaging reporter gene.[47] To date, there have been a number of studies investigating the application of reporter gene expression imaging coupled with gene therapy of prostate cancer, particularly using viral vector based gene therapy systems and bioluminescence imaging.[48-51] Bioluminescence imaging has proved to be a very useful technique for monitoring gene expression in preclinical models of gene therapy. One of the most useful applications has been the monitoring of therapeutic gene expression in targeted tissues in disease models, while assessing the effectiveness and safety of systems of gene therapy delivery.

Li and coworkers employed a CCD imaging system to monitor prostate-specific adenoviral vector mediation of long-term, sustained expression of *Fluc* in living prostate cancer mouse models with the goal of assessing the effectiveness and safety of systems of gene therapy delivery.[50] Iyer and coworkers used a 2-step transcriptional amplification (TSTA)-based lentiviral vector (LV-TSTA) to demonstrate specific targeting of prostate tumors in vivo after systemic administration of lentivirus. They showed that noninvasive imaging using such vectors could be useful for monitoring long-term gene expression in gene therapy applications.[49] Sato and coworkers used a similar prostate-specific TSTA method, with the aim of developing an effective and safe gene-based treatment for prostate cancer. Because very limited treatment options are available for recurrent hormone refractory prostate cancer (HRPC), the aim was to assess whether PSA promoter-based TSTA gene therapy would be functional in HRPC. It was shown that the prostate-specific TSTA gene expression vectors exhibited robust activity in HRPC as well as androgen-dependent tumors. They concluded that such vector-based gene therapy coupled to reporter gene imaging would be a promising therapeutic option to develop for treating patients with recurrent disease.[51]

Although bioluminescence has been the predominant modality used in experimental reporter gene imaging of prostate cancer gene therapy, others have demonstrated the use of alternative reporter gene imaging techniques such as PET and SPECT.[52-54] For example, Pantuck and coworkers developed a prostate cancer tumor model amenable to noninvasive imaging using PET, based on expression of the HSV1-*tk* reporter gene.[53] The reporter signal was imaged using ^{18}F-FDG and ^{18}F-FHBG micro-PET. This group described the feasibility of reporter gene imaging of adenoviral delivered HSV1-*tk* suicide gene therapy of prostate cancer. They proposed that this technique could be used to noninvasively monitor the location, duration and extent of gene expression, thus contributing to the safety of clinical gene therapy protocols. It could be used as well to noninvasively image prostate cancer xenograft response to experimental gene therapy.

The marriage of noninvasive reporter gene expression imaging to cytotoxic gene therapy provides a promising strategy to monitor gene therapy techniques both within experimen-

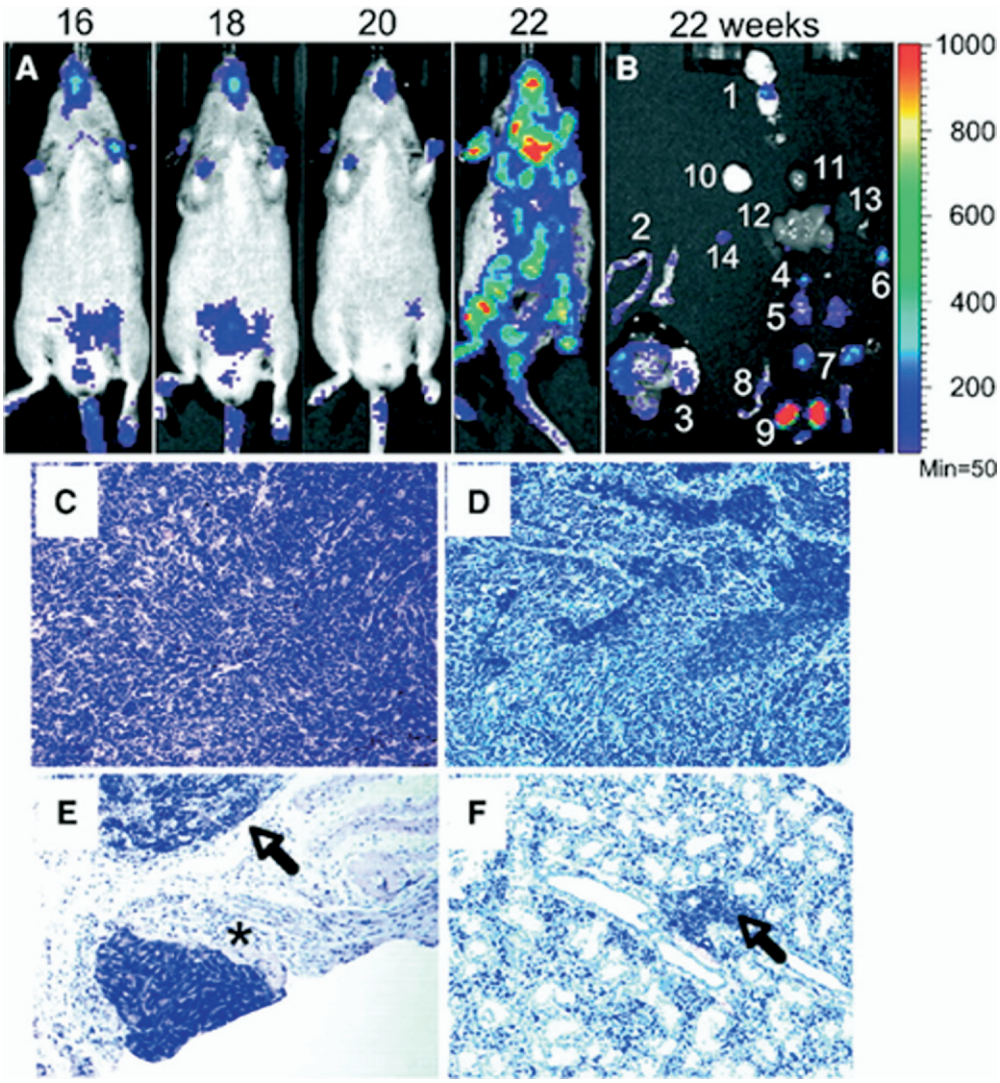

Figure 3 Detection of metastasis in living EZC3-TRAMP mice. (A) Mouse 2153 imaged from 16 to 22 weeks at equivalent conditions. (B) Mouse 2153 imaged ex vivo on dissection at equivalent color scale. 1, salivary gland; 2, cecum and colon; 3, urogenital tract; 4, adrenal gland; 5, kidney; 6, mesenteric lymph node; 7, para-aortic draining lymph node; 8, epididymis; 9, testes; 10, lung; 11, heart; 12, liver; 13, spleen; 14, pancreas. (C-F) Hematoxylin and eosin staining of draining para-aortic lymph node (C), mesenteric lymph node (D), pancreas (E), and salivary gland (F) imaged in (B). Arrows indicate tumor lesions. *Pancreatic tissue. (Reproduced with permission from Seethammagari et al.[36])

tal research and future clinical trials. To this aim, Johnson and coworkers postulated that cancer gene therapy based on tissue-restricted expression of cytotoxic transgenes should achieve superior therapeutic index over an unrestricted method.[52] They compared the therapeutic effects of a highly augmented prostate-specific gene expression method with another approach driven by a strong constitutive promoter instead. The imaging reporter gene (*Fluc*) and the cytotoxic gene (HSV1-*tk*) were delivered by adenoviral vectors injected directly into human prostate tumors grafted in SCID mice. Along with serial bioluminescence imaging, PET and computerized tomography were also employed to image the therapeutic effects. These techniques demonstrated restriction of gene expression to the tumors where the prostate-specific vector was used. Another study, to aid in the design of a phase I gene therapy trial in patients with prostate cancer, determined the feasibility of using the human sodium iodide symporter (hNIS) and SPECT as a reporter gene imaging technique to study the dynamics of adenoviral transgene expression in a large animal (canine) tumor model. The dosimetric characteristics of the reporter gene system was also assessed following intravenous administration of radioactive sodium pertechnetate ($Na^{99\ month}TcO_{(4)}$). A replication-competent Ad5-yCD/mut*tk*(*sr39*) rep-*hNIS* adenovirus was injected into the canine prostate gland and into a canine soft tissue sarcoma for dosimetry and imaging purposes, respectively. The study concluded that up to five imaging procedures could be safely performed in humans after intraprostatic injection of the Ad5-yCD/mut*tk*(*sr39*)rep-*hNIS* adenovirus and the hNIS reporter gene system could be used

to study the dynamics of adenoviral gene therapy vectors in large animal tumors.[54]

MRI techniques have also been shown to detect and monitor reporter gene expression, highlighting its potential use in evaluating gene therapy trials of prostate cancer. Lui and coworkers designed a ^{19}F-based nuclear magnetic resonance (NMR) approach to reveal *lacZ* gene expression by assessing beta-galactosidase (β-gal) activity in vivo. The substrate 2-fluoro-4-nitrophenyl beta-D-galactopyranoside (OFPNPG) is readily hydrolyzed by β-gal with a corresponding decrease in the ^{19}F-NMR signal from OFPNPG and the appearance of a new signal shifted 4 to 6 ppm upfield from the aglycone 2-fluoro-4-nitrophenol (OFPNP). It was shown as proof of principle that ^{19}F-NMR spectroscopy and ^{19}F chemical shift imaging could be used to detect reporter gene expression in prostate cancer and so provide a potential means of imaging gene therapy in future clinical trials.[55]

Imaging of Transgenic Models of Spontaneous Disease

The use of transgenic models provides a valuable medium in which to replicate molecular, physiological and histological features of human disease. Currently, the mouse model provides the most accurate representation of human disease in vivo, because, as well as having similar anatomy and physiology, its genome can be easily manipulated. By using transgenic mice, there have been significant advances in our understanding of prostate cancer pathogenesis, growth and potential therapeutic strategies.[56-62] The advantages of reporter gene imaging discussed above can be harnessed fully in transgenic mice models of prostate cancer, as they provide an environment reflective of true in vivo pathophysiologic conditions.

Xie and coworkers described a novel bigenic, transgenic model allowing both ex vivo and in vivo imaging of the prostate.[63] Both Fluc and enhanced green fluorescent protein were targeted to the prostate epithelium using a composite human kallikrein 2 (hK2)-based promoter (hK2-E3/P), allowing both bioluminescence and fluorescence imaging of the prostate. Using this transgenic model, it was shown that the hK2-E3/P promoter directed strong prostate specific expression and thus demonstrated the usefulness of such transgenic models to further study neoplastic prostate disease models, looking at for example, tumor growth, androgen signaling, metastases and novel therapies such as gene therapy.

Lyons and coworkers developed a novel transgenic murine model incorporating the *Fluc* gene in the epithelium of all lobes of the prostate gland, allowing in vivo imaging of both androgen dependant and androgen independent prostate tumor models using bioluminescence imaging.[64] The data provided evidence that the Fluc reporter could allow bioluminescence imaging of androgen independent prostate tumors in transgenic mice, since prostate tissue in these mice continued bioluminescence following androgen depletion. As proof-of-principle, this group also demonstrated that these techniques could potentially also be used to image spontaneous tumorogenesis and response to hormone ablation in these models.

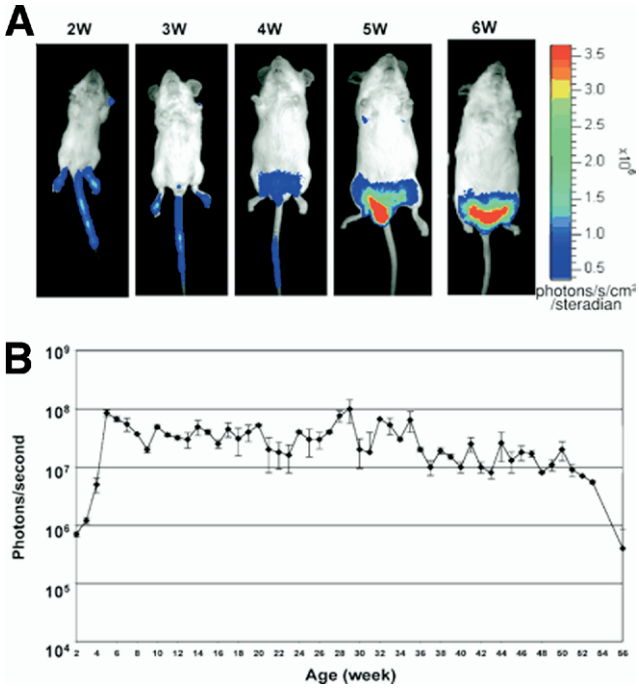

Figure 4 Developmental regulation of PSA-Luc expression. (A) Bioluminescent images of a representative individual mouse. The animals were measured at 1-week intervals from 2 to 6 weeks (W) old. Signals were adjusted to the same color scale for the entire time course. (B) Quantification of photons emitted from the lower abdomen of PSA-Luc male mice from 2 to 56 weeks old. Data are presented as average photon count/s of 3 mice, and standard deviation. (Reprinted from Hsieh et al.[65] © 2005 Society for Endocrinology. Reproduced by permission.)

In addition to prostate cancer growth and development, other studies have used transgenic murine models and bioluminescence *Fluc* gene expression to noninvasively image prostate cancer metastases to bone and soft tissues. Hsieh and coworkers previously developed a bioluminescence mouse model with Fluc activity restricted to the prostate gland under the control of a PSA promoter that could be used on a real-time basis in live animals to investigate the development and responsiveness of the prostate gland to therapy, such as exogenoulsy administered androgen (Fig. 4).[65] The group then recently used a bioluminescence bigenic mouse model combining sPSA-*Fluc* reporter gene with TRAMP (transgenic adenocarcinoma mouse prostate)-*Fluc* reporter gene, to assess the feasibility of generating bigenic mice to evaluate noninvasively the metastatic potential of prostate tumors.[66]

Imaging of Molecular Interactions or Events

Some interesting variations on standard reporter gene assays described above have also been adapted recently for imaging of molecular interactions in living subjects. More specifically, the emerging ability to noninvasively image protein-protein interactions (eg, using split reporter complementation and reconstitution strategies) has important implications for a

wide variety of biological research endeavors, drug discovery, and molecular medicine.[67]

As well as providing the means to assess the general behavior of the prostate cancer cells in question, reporter gene imaging using gene marked cells can be used to indirectly image subcellular components such as enzymes and proteins important in the pathogenesis of prostate cancer. Ilagan and coworkers developed a gene expression-based imaging system that detects and quantifies mitogen-activated protein kinases (MAPK) activity in prostate cancer tumors implanted into severe combined immunodeficient mice.[68] The imaging technology used a modified version of a TSTA system where the tissue specificity of gene expression is imparted by an enhanced version of the PSA regulatory region that expresses GAL4-ELK1. GAL4-ELK1 conferred MAPK specificity by activating *FLuc* when the Ets-like transcription factor (ELK) 1-activation domain is phosphorylated by MAPK. The TSTA-ELK1 system was validated by analyzing its response to epidermal growth factor treatment in transfected tissue culture cells and in adenovirus (AdTSTA-ELK1)-injected prostate cancer xenograft tumors. The authors measured MAPK activity in 2 well-characterized xenograft models, CWR22 and LAPC9. Although no significant differences in MAPK levels were detected between androgen-dependent and androgen-independent xenografts, the CWR22 models displayed significantly higher levels of AdTSTA-ELK1 activity versus LAPC9. Western blots of tumor extracts showed that the elevated imaging signal in CWR22 xenografts correlated with elevated levels of phosphorylated extracellular signal-regulated kinase 1/2 but not p38 or c-Jun NH^2-terminal kinase. The authors concluded that a gene expression-based optical imaging system can accurately detect and quantify MAPK activity in live animals.

Molecular imaging of interacting protein partners or protein trafficking in mice could pave the way to functional proteomics in whole animals, the assessment of dysfunctional signaling networks in diseased cells, and provide a tool for evaluation of new pharmaceuticals targeted to modulate protein-protein interactions and protein translocation.[67] Nucleocytoplasmic trafficking of functional proteins plays a key role in regulating gene expressions in response to extracellular signals, particularly in relation to AR activity in prostate cancer. To study AR translocation, Kim and coworkers have developed a genetically encoded bioluminescence indicator for imaging the nuclear trafficking of target proteins in vivo.[69] The principle is based on reconstitution of split fragments of Renilla reniformis (Rluc) by protein splicing with a DnaE intein (a catalytic subunit of DNA polymerase III). A target cytosolic protein fused to the N-terminal half of Rluc is expressed in mammalian cells. If the protein translocates into the nucleus, the Rluc moiety meets the C-terminal half of Rluc, and full-length Rluc is reconstituted by protein splicing. The authors demonstrated quantitative cell-based in vitro sensing of ligand-induced translocation of the AR, which allowed high-throughput screening of exogenous and endogenous agonists and antagonists. Furthermore, the indicator enabled noninvasive in vivo imaging of AR translocation in the brains of living mice with a CCD camera. These rapid and quantitative analyses in vitro and in vivo may provide a wide variety of applications for screening pharmacological or toxicological compounds and testing them in living animals.

Future Outlook

The merger of molecular biology and medical imaging is facilitating rapid growth of novel molecular imaging techniques by providing methods to monitor an ever increasing number of cellular/molecular events adapted from conventional molecular assays, including reporter gene assays. With regard to prostate cancer, further progress in visual representation, characterization, quantification, and timing of these biological processes in living subjects could create unprecedented opportunities to complement available in vitro or cell culture methodologies in order (1) to characterize more fully the biology of intracellular events, for instance, protein–protein interactions, in the context of whole-body physiologically authentic environments and (2) to accelerate the evaluation in living subjects of novel drugs, for example, those that that promote or inhibit signal transduction pathways.

Further progress in reporter gene imaging will also provide us with the ability to perform multiplex imaging, that is, simultaneous imaging of multiple molecular events in one population of cells in living subjects. This may be attainable by combining 2 or more of the aforementioned strategies for gene marking and imaging cellular trafficking with those entailing linked expression of an imaging gene to an endogenous promoter, or to an exogenous therapeutic gene. This could allow one reporter to reveal the spatial distribution of cells and whether they have reached a specific target, and another reporter to indicate whether a certain gene becomes upregulated at this site or if a more complex interaction occurs. These endeavors will be aided by the availability of multiple fusion reporter constructs (eg, those that combine PET/biolumoinescence/fluorescence imaging capabilities in one gene),[70] the use of which should accelerate the validation of reporter gene approaches developed in cell culture for translation into preclinical models and subsequent clinical imaging of prostate cancer. With continued rapid advancements in this field, the experimental and clinical disciplines devoted to the study of prostate cancer stand to gain considerably from noninvasive molecular imaging of the expression of multiple fused reporter genes using multiple imaging modalities.[8] These approaches are likely to play an increasingly important role in defining molecular events in the field of cancer biology, cell biology, and gene therapy in prostate cancer.

It is anticipated that many of the experimental applications of reporter gene expression imaging reviewed above, will eventually translate from animal research to clinical practice.[71] However, there are theoretical and practical challenges in attempting to translate these research strategies to clinical applications in humans.[21] The hurdles to be overcome include the need to address issues of probe biocompatibility, overcoming physiological and morphological barriers to the delivery of genes and probes, amplification of low level in

Table 1 Features of Molecular Imaging Modalities Used in Reporter Gene Imaging

Imaging Technique	Advantages	Disadvatages
Bioluminescence optical imaging	• Very high sensitivity • High throughput • Very versatile • Cheap	• Very low spatial resolution • Only planar imaging, not tomographic • Surface weighted images owing to light scatter and absorption • Semiquantitative imaging data • Mass amount of probe required (may be toxic) • Not an established clinical modality
MRI	• Very high spatial resolution • Tomographic imaging • Widely available, established clinical modality	• Low sensitivity • Mass amount of probe required (may be toxic)
PET and SPECT	• High sensitivity • Fully quantitative imaging data • Tomographic imaging • Nanogram amount of probe required (nontoxic and safe) • Established clinical modalities	• Low spatial resolution • Probes for using HSV1-TK gene do not cross the blood-brain barrier • D2R gene normally expressed in basal ganglia interferes with image interpretation when using this reporter system

PET, positron emission tomography; SPECT, single-photon emission computed tomography.

vivo signals of biological events, and the development of imaging platforms with sufficiently high sensitivity and spatial and temporal resolution.

Gene therapy is an important discipline where current experimental endeavors are being scrutinized and further refined for potential clinical translation in the near future. The hope is that reporter gene imaging research in prostate cancer may ultimately integrate with these gene therapy techniques to allow the prospect of noninvasive imaging and monitoring of transgene expression, disease progress, and response to therapy. Although we are still some way in harnessing the full potential of gene therapy in the clinic, much progress has been made in overcoming many of the obstacles.[72] Encouragingly, using murine prostate cancer models, the ability of reporter gene imaging to successfully monitor and track transgene expression and gene therapy has been convincingly demonstrated in the experimental setting as exemplified above. The challenge remains to generate disease- or site-specific imaging strategies that are common to both gene therapy and reporter gene imaging. It is likely that experimental work will focus on both transductional targeting of the vector and restriction of reporter gene expression solely to the target in question.

Another practical hurdle faced in translating reporter gene imaging of prostate cancer from laboratory to clinic is the need for sufficient imaging probe to reach the target in vivo to achieve satisfactory specificity. Both PET and SPECT have advantage over MRI and optical imaging, in that they only require trace quantities of molecular probe to obtain images. These trace (nonpharmacological) nanogram levels of molecular probe are known to be safe in humans. Yaghoubi and coworkers demonstrated good kinetics, biodistribution, stability, dosimetry, and safety of ^{18}F-FHBG in healthy human volunteers.[73,74] This reporter probe is used for imaging expression of the HSV1-*tk* reporter gene and could be used in the future for imaging of patients undergoing HSV1-*tk* suicide gene therapy. Initial clinical experience using this reporter gene/probe combination has been achieved in glioma[75] and liver cancer gene therapy.[76]

Currently both optical imaging and MRI have limitations hindering their potential for clinical use, as outlined in Table 1. With optical imaging the main drawbacks are poor surface detection of light and poor spatial resolution, whereas for MRI these include poor sensitivity and the requirement for large quantities of probes. PET and SPECT imaging therefore currently provide the best compromise and potential for clinical translation of molecular reporter gene imaging of prostate cancer owing to their advantages, as summarized in Table 1. Further development of noncompetitive partnerships between current established imaging modalities such as PET/CT and MRI/MRIS, and molecular imaging of reporter gene expression (eg, using bioluminescence or PET) would certainly extend the scope and value of experimental research in prostate cancer and hopefully expedite the future clinical implementation of reporter gene imaging. As an example of such a complementary association of imaging techniques, Lee and coworkers recently investigated the potential use of an MRI-based functional diffusion map as an imaging biomarker for assessing early treatment response in a preclinical murine model of metastatic prostate cancer. Optical bioluminescence imaging using Fluc was used as a screening tool to select those subjects that exhibited metastatic disease in the first instance before they were further imaged using MRI.[77]

Molecular imaging using reporter genes is emerging as a valuable tool for monitoring gene expression in laboratory animals and humans. Further development of more sensitive and selective reporters, combined with improvements in detection technology will consolidate the position of reporter gene imaging as a versatile method for understanding of intracellular biological processes and the underlying molecular basis of prostate cancer, as well as potentially establishing a future role in the clinical management of patients afflicted with this disease.

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