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Cancer Imaging: Gene Transcription-Based Imaging and Therapeutic Systems

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Abstract

Molecular-genetic imaging of cancer is in its infancy. Over the past decade gene reporter systems have been optimized in preclinical models and some have found their way into the clinic. The search is on to find the best combination of gene delivery vehicle and reporter imaging system that can be translated safely and quickly. The goal is to have a combination that can detect a wide variety of cancers with high sensitivity and specificity in a way that rivals the current clinical standard, positron emission tomography with [^{18}F]fluorodeoxyglucose. To do so will require systemic delivery of reporter genes for the detection of micrometastases, and a nontoxic vector, whether viral or based on nanotechnology, to gain widespread acceptance by the oncology community. Merger of molecular-genetic imaging with gene therapy, a strategy that has been employed in the past, will likely be necessary for such imaging to reach widespread clinical use.

Keywords

Cancer imaging; Tumor-specific promoter; Theranostics

1. Introduction: Molecular Imaging for Cancer Detection

1.1. Direct Imaging of Cancer

Current non-invasive imaging techniques utilized for cancer detection and diagnosis can be categorized into two groups: direct and indirect strategies as previously defined (Blasberg and Tjuvajev, 2003). The direct strategy is based on direct interactions between molecular targets and imaging agents that are specifically designed for the target. The molecular targets are often cell markers that are over-expressed or exclusively expressed in malignant cells. One example is our long-standing effort to develop multi-modal small molecule-based imaging agents with high affinity to prostate-specific membrane antigen (PSMA) (Chen et al., 2008; Foss et al., 2005). PSMA is a well-characterized cancer cell surface protein, which is highly expressed in prostate cancer tissues and in most non-prostate solid tumor neovasculature. A series of urea derivatives with high affinity to PSMA have been labeled with radioisotopes, fluorescent dyes or gadolinium chelates for radionuclide, optical, or magnetic resonance imaging, respectively. Another example of the direct imaging strategy includes the use of [^{18}F]fluorodeoxyglucose (FDG), a glucose analog labeled with the positron-emitting radioisotope, ^{18}F , and the most widely used imaging agent for cancer staging.

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[¹⁸F]FDG is concentrated specifically within malignant cells based on the up-regulation of glucose transporter 1 (GLUT1) on the cancer cell surface. Its cancer-specific uptake also relies upon the Warburg effect, i.e., the increased glucose metabolism of cancer cells.

1.2. Reporter Gene Expression-Based Imaging for Cancer Detection: Molecular-Genetic Imaging

The indirect strategy, often called molecular-genetic imaging, is based on cancer cell-specific reporter gene transcription. The advantage of the indirect strategy is that well-characterized reporter-imaging agent pairs can be applied to different systems and types of disease while the direct strategy requires the development of a specific imaging agent for each molecular target. For example, the most widely used reporter-probe pairs include: (i) firefly luciferase (FLuc)–D-luciferin and renilla luciferase (RLuc)–coelenterazine pairs for *in vivo* bioluminescence imaging; (ii) wild type or mutant herpes simplex virus type 1 thymidine kinase (HSV1-TK)–radiolabeled nucleoside analogs; and, (iii) the sodium iodide symporter (NIS)–¹²⁴I or ¹²³I pairs for positron emission tomography (PET) or single photon emission computed tomography (SPECT), respectively. Fluorescent proteins are reporters, which do not require specific substrates or imaging agents to produce light output. Recent development of diverse red fluorescent protein (RFP) variants with near infrared emission wavelengths has broadened their application potential for *in vivo* imaging by significantly improving tissue penetration (Shaner et al., 2004). Other advantages over the direct imaging strategy are the potentially increased accuracy and sensitivity of cancer detection, since the indirect strategy can be viewed as a dual step-imaging process. That is especially true for enzyme-based reporters. As the first step, the transcription and expression of reporter genes proceeds – selectively within cancer cells eliminating background signal. Second, the imaging probe specific for the reporter is administered. The resulting light emitting enzymatic reactions or the accumulation of radioactive probes within reporter-expressing cells provides the readout of the signal. Sensitivity derives from the mechanism of signal amplification inherent to enzymatic reporter genes, where the enzyme can turn over many radioactive substrates. In_[HB1] term of translational potential, HSV1-TK and NIS have several advantages over FLuc and RLuc. PET and SPECT are imaging modalities that are currently used for patients, and the tracer pairs for HSV-1TK and NIS are already used in the clinic. On the other hand, bioluminescent light signals from luciferase reporters have the wavelengths below 600 nm with poor tissue penetration. Their substrates, D-luciferin and coelenterazine cannot be used for patients. In addition, current technology does not provide imaging devices equipped with charge-coupled device (CCD) cameras, which are capable of imaging large subjects other than small laboratory animals such as rodents and rabbits.

1.3. Components of Reporter Gene Transcription-Based Cancer Imaging Systems

Required components of a molecular-genetic imaging system include: (i) gene promoter/enhancer that regulates reporter gene expression; (ii) reporter genes (Bhaumik, 2011; Penheiter et al., 2012; Ray, 2011); (iii) delivery vector/vehicle (Haisma and Bellu, 2011; Lam and Dean, 2010; Nowrouzi et al., 2011) for promoter and reporter gene constructs into animal models and ultimately into patients; and, (iv) imaging probes specifically paired to the reporters. To ensure that the reporter expression occurs in a cancer-specific manner, which is the primary requirement for success, two options are possible. First, one can employ a promoter/enhancer element that regulates downstream gene expression in a cancer-specific manner. Second, gene delivery vehicles that have tumor-targeting moieties for a constitutively active promoter can be used to drive reporter expression specifically within malignant cells. However, here we will concentrate on the former where the cancer-specificity originates from the promoters employed. Key criteria for selecting an appropriate promoter for cancer imaging include: (i) cancer specificity that ensures minimal background in normal tissues; and, (ii) strong promoter activity to provide high tumor to background

ratios (Figure 1). Here we will provide an overview of the literature related to molecular-genetic imaging systems for cancer detection by focusing on the choice of promoters.

The application of molecular-genetic imaging for cancer detection has relatively a short history, yet an increasing number of strategies are being implemented, as shown in Figure 2. As described previously, the choice of promoters that control the selective expression of reporter genes is the key to success for this technique. In the following sections we will discuss a diverse set of gene promoters that have been used for molecular-genetic imaging throughout the last decade.

2. Promoters that Regulate Reporter Gene Transcription/Expression

2.1. Organ-specific promoters

2.1.1. Liver-specific promoters—The α -fetoprotein (AFP) promoter that consists of the basal promoter and enhancer I region was constructed (Willhauck et al., 2008) and tested preclinically for its specificity for hepatocellular carcinoma cells. Most recently Klutz, *et al.* reported the development of novel imaging and radiotherapy systems for hepatocarcinoma based on the mouse AFP promoter (Klutz et al., 2011). They utilized a replication-incompetent adenoviral vehicle for intratumoral local administration into human hepatocarcinoma HepG2 xenografts. AFP promoter-driven NIS served as both reporter and therapeutic gene paired with a γ emitter ^{123}I and β emitter ^{131}I , respectively. This and several other previous reports demonstrated the potential of using the AFP promoter for imaging and therapy of hepatocellular carcinoma.

Another example of a hepatocarcinoma-specific promoter is the hepatocarcinoma-intestine-pancreas (*HIP*) gene promoter. Herve, *et al.* (Herve et al., 2008) applied a very similar experimental scheme to that of Klutz, *et al.* (Klutz et al., 2011). *HIP*, also known as pancreatitis-associated protein-1 (PAP1) or RegIIIa, was reported to be up-regulated in most cases of human hepatocellular carcinoma (Christa et al., 1999). Adenoviral vectors containing the rat *HIP* promoter upstream of a NIS encoding sequence were injected intraperitoneally into rats harbouring carcinogen-induced hepatocellular carcinoma. Again through NIS, [$^{99\text{m}}\text{Tc}$]TcO $_4^-$ (i.e. pertechnetate) and ^{131}I were used for imaging and therapy, respectively, of this model of hepatocellular carcinoma.

In addition to AFP and *HIP* gene regulatory sequences, murine albumin promoter and human α 1-antitrypsin (hAAT) have been used as liver-specific promoters that regulate gene expression in small animal models of hepatocellular carcinoma (Chen et al., 2006; Lam et al., 2007).

2.1.2. Prostate-specific promoters—The prostate-specific antigen (PSA) promoter has been most extensively tested for its prostate cancer-specific promoter activity in various preclinical models of prostate cancer. Wu *et al.* pioneered use of the PSA promoter for prostate-specific imaging and gene therapy. They identified and constructed the chimeric promoter PSE-BC by inserting two tandem repeats of PSA core enhancer sequences and reducing the distance between the cores and the proximal promoter region, which resulted in a significantly improved promoter activity compared to the native PSA promoter and enhancer region (Wu et al., 2001). Iyer, *et al.* further improved the transcriptional activity driven by the PSA proximal promoter and enhancer sequence by developing a two-step transcriptional amplification (TSTA) system (Iyer et al., 2001; Wu et al., 2001). As the name implies, the first step of the TSTA system results in prostate-selective expression of the GAL4-VP16 fusion protein under the control of the PSA promoter. In the second step, the GAL4-VP16 fusion protein in turn binds to GAL4 response elements, which switch on the transcription of reporter genes as an amplified end product. Burton, *et al.* demonstrated that

AdTSTA-sr39tk, an adenoviral vectors expressing HSV1-sr39TK under the control of the PSE-BC promoter amplified through TSTA, could detect sentinel lymph node metastasis in an orthotopic murine model of prostate cancer (Burton et al., 2008). In addition, Pouliot, *et al.* suggested the potential application of AdTSTA-sr39TK for the detection of prostate cancer in a canine model after intraprostatic administration (Pouliot et al., 2011).

Recently, Jiang, *et al.* reported a PSES (prostate-specific enhancing sequence)-based imaging system for the detection of castration-resistant prostate cancer (CRPC) (Jiang et al., 2011). PSES was developed by fusing androgen-inducible PSA enhancers with androgen-suppressive prostate-specific membrane antigen (PSMA) enhancer elements to create an androgen-independent promoter (Lee et al., 2002). The TSTA system was applied to multiply the transcriptional activity by PSES.

In addition to PSA and PSMA gene transcriptional regulatory elements, androgen-regulated proteins, probasin (Trujillo et al., 2010) and Kallikrein 2 (Xie et al., 2004) gene promoters were employed to drive prostate-specific reporter expression.

2.1.3. Pancreas-specific promoter—Cholecystokinin type A receptor (CCKAR) has been considered as a potential cancer marker that is over-expressed in human pancreatic ductal adenocarcinomas (PDAC) compared to normal ductal tissues (Weinberg et al., 1997). Li, *et al.* previously suggested the potential of CCKAR gene promoter as a cancer-specific promoter for gene therapy targeting pancreatic cancer (Li et al., 2006). Xie, *et al.* further improved the strength of the CCKAR promoter by adopting the TSTA system and demonstrated that this promoter can drive downstream gene expression in a pancreatic cancer-specific manner in mouse xenograft models (Iyer et al., 2001; Xie et al., 2007). They developed a plasmid DNA (pDNA) construct containing the CCKAR promoter upstream of the FLuc reporter gene and administered it as a complex of pDNA and cationic liposome composed of 1,2-dioleoyl-3-trimethyl-ammonium propane (DOTAP):cholesterol intravenously into an orthotopic mouse model. When systemically injected into the orthotopic AsPC-1 model, *in vivo* bioluminescence imaging showed that FLuc expression was confined to the sites of tumor.

2.2. Cell type-specific promoters

2.2.1. Melanogenic cell-specific promoter—Tyrosinase is the critical enzyme in melanin biosynthesis and known to be exclusively expressed in melanogenic cells. Accordingly, the tyrosinase promoter is active only in melanogenic cells, for example, melanocytes that are specialized cells involved in melanogenesis (Riley, 1997). Lillehammer, *et al.* suggested the potential of the tyrosinase promoter for imaging and therapy for melanotic melanomas (Lillehammer et al., 2005). They developed an adenoviral construct containing enhanced green fluorescence protein (EGFP), the transcription of which is regulated by 2hE-hTyrP, consisting of the human minimal tyrosinase promoter and two human enhancer elements. When adenovirus was locally administered into the melanotic melanoma FEMX-1 xenograft, 2hE-hTyrP drove EGFP expression on a comparable level to the most commonly used promoter for its constitutive activity, the cytomegalovirus (CMV) promoter.

2.2.2. Schwann cell-specific promoter—Myelin protein zero (P0) is known to be exclusively expressed in schwann cells (Lemke and Axel, 1985). Prabhakar, *et al.* applied the P0 gene promoter to develop gene therapy for schwannoma by using pro-apoptotic enzyme, caspase-1 (ICE) as a therapeutic gene (Prabhakar et al., 2010). They showed that the activity of the P0 promoter was specific in Schwann cells, compared to fibroblast and neuroblastoma cell lines. This result suggests that P0 promoter-based gene therapy for

schwannoma might avoid injury to associated nerves by causing minimal off-target effects. The HSV amplicon was used as a gene delivery vector in this report. Its specific activity for schwannoma cells was confirmed in a HEI-193 schwann cell xenograft model by *in vivo* bioluminescence imaging.

2.3. Promoters tested for their broad cancer specificity

There have been successful examples of using organ- or cell type-specific promoters but their application is limited to cancer of certain tissue origins and subtypes. Therefore, researchers have been searching for promoters with universal cancer selectivity to achieve broad applications.

Some of those examples include human telomerase reverse transcriptase (hTERT), survivin and carcinoembryonic antigen (CEA) promoters. One example of the most successful applications of hTERT promoter is OBP-401 (Kishimoto et al., 2006). OBP-401 (TelomeScan®) is a replication-competent adenovirus variant where the hTERT promoter element drives the expression of viral *E1* genes, which are required for the viral replication. To monitor the viral replication, the GFP gene is inserted under the CMV promoter in the same viral vector. Therefore, OBP-401 replicates and, in turn, expresses GFP only in cells with the active telomerase, i.e. malignant cells (Kishimoto et al., 2006). This system has been tested in murine xenograft models of colon, liver and prostate cancer (Kishimoto et al., 2006; Kishimoto et al., 2009a; Kishimoto et al., 2009b). The efforts to apply OBP-401 for an *ex vivo* diagnostic tool to detect circulating tumor cells in patient blood samples have been recently reported (Kim et al., 2011; Kojima et al., 2009).

The survivin promoter is another example of a promoter that has been suggested to have cancer specificity. Ahn, *et al.* utilized the survivin promoter to drive the simultaneous expression of (i) a reporter gene, FLuc; and, (ii) a pro-apoptotic gene, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) for therapy (Ahn et al., 2011). The TSTA system was applied to enhance its promoter activity (Iyer et al., 2001; Wu et al., 2001). The tumor selectivity of the survivin promoter was demonstrated in a rodent hepatocellular carcinoma model. Huang, *et al.* employed the survivin promoter to control the expression of NIS for the ¹³¹I-based radiotherapy (Huang et al., 2011). By using pertechnetate, NIS expression in PC3 prostate cancer xenografts was able to be imaged in mice. In both studies, adenoviral vectors were employed to deliver the genes to the target tumors. Very similar strategies for cancer imaging and therapy were developed by employing CEA promoter (Qiao et al., 2002; Spitzweg et al., 2007). The expression of HSV1-TK (Qiao et al., 2002) and NIS (Spitzweg et al., 2007) were driven by CEA promoter in murine xenograft models of breast and medullary thyroid cancer, respectively.

Inhibitor of DNA binding-1 (Id-1) is a helix-loop-helix (HLH) protein and transcriptional regulator. Id-1 functions as a dominant negative regulator of basic HLH transcription factors by binding to them and forming inactive heterodimers. Up-regulated Id1 expression was observed in various tumor types including breast, non-small cell lung, ovarian cancer and melanoma and within the vasculature of certain tumors (Perk et al., 2006; Sikder et al., 2003). Warram, *et al.* employed the Id-1 promoter and developed a dual reporter adenoviral vector containing secreted embryonic alkaline phosphatase (SEAP) and a red fluorescent protein mCherry downstream of the Id-1 promoter. Only human breast cancer cell lines were tested for Id-1 promoter activity to drive dual reporters for simultaneous diagnostic screening and *in vivo* imaging (Warram et al., 2011). However, the broad expression profile of Id-1 in malignant tissues of diverse origins and their associated vasculature suggests broader applications.

Using an adenoviral vector Huyn, *et al.* developed a mucin-1 (MUC1) promoter-driven imaging system based on the expression of FLuc (Huyn et al., 2009). They employed the TSTA system to amplify MUC1 promoter activity. This imaging strategy detected lymph node and liver metastases from breast cancer after peritumoral or systemic administration, respectively, in mouse models. MUC1 has been reported as a biomarker indicative of poor prognosis in breast, prostate, lung and thyroid cancers (Kufe, 2009). Therefore, the MUC1 promoter is also expected to be applicable for imaging of a diverse set of cancers.

3. Toward a cancer-selective promoter with broad applicability: PEG-3 promoter

The promoters we overviewed in the previous section share one common feature: they are the transcriptional regulatory elements of biomarkers found in human cancer cells. On the other hand the progression elevated gene-3 (*PEG-3*) promoter can be distinguished by its unusual origin of its selectivity for a wide variety of malignancies. The *PEG-3* promoter is approximately 500 bp-long minimal functional region of rodent *PEG-3* gene promoter. *PEG-3* was first identified as an up-regulated transcript during tumor progression of mutant type 5 adenovirus-transformed rodent embryonic fibroblasts (Su et al., 1997). *PEG-3* is a truncated form of a tumor suppressor gene, growth arrest and DNA damage-inducible protein 34 (*GADD34*), which functions as a dominant negative mutant GADD34. This mutation process is known to be unique for the disease progression in rodents. When tested in panels of human cell lines, the *PEG-3* gene promoter showed cancer-specific activity in a variety of tissues, including prostate, breast and pancreas (Su et al., 2005). In addition to its broad specificity for cancer, the most interesting characteristic of the PEG-3 promoter is that there is no homologous sequence in the human genome. Rather than its activity depending upon the transcriptional activity of a single gene, the PEG-3 promoter functions as a sensor for the cellular environment of cancer cells. Two transcription factors, AP-1 and PEA-3, are known to be key regulators for the PEG-3 promoter activity. However, tentative binding sites for other transcription factors are predicted by bioinformatic tools, and may provide further insight into the cancer selective activity of PEG-3 promoter.

Recently we have employed the PEG-3 promoter for imaging metastasis *in vivo* (Bhang et al., 2011). We chose the PEG-3 promoter based on: (i) its universal specificity for cancer regardless of the tissue origin and subtypes shown in the previous *in vitro* studies; (ii) the lack of its sequence homology within human genome, thereby minimizing nonspecific gene expression in normal tissues; and, (iii) its strong promoter activity, comparable to that of the SV40 early promoter. Through bioluminescence imaging with FLuc and radionuclide imaging based on HSV1-TK and [¹²⁵I]FIAU, we were able to demonstrate that PEG-3 promoter drives the expression of reporters selectively in metastatic tissues, enabling the detection of micrometastasis in experimental metastasis mouse models. We developed a pDNA construct containing PEG-3 promoter upstream of FLuc or HSV1-TK reporter genes and administered it as a complex of pDNA and polyethylenimine (PEI), systemically. The use of an untargeted, inert delivery vehicle and the highly specific activity of PEG-3 promoter enabled systemic delivery, which we believe to be important for clinical translation.

4. Toward theranostics: combining imaging with gene therapy

Molecular-genetic imaging has been performed in the clinic since 2001 (Freytag et al., 2007; Jacobs et al., 2001), however, only with delivery of low molecular weight radiopharmaceuticals and only when merged with ongoing gene therapy. Nanoparticle delivery of molecular-genetic imaging systems has not yet reached the clinic. For molecular-genetic imaging to be adopted extensively for clinical use, it will likely have to be linked to

therapy, and several important hurdles will have to be overcome. For example, there have been several well-publicized setbacks for gene therapy – on which the fate of molecular-genetic imaging likely rests. In one case the viral gene delivery vector was the culprit, but causing leukemia in several patients treated for X-linked severe combined immunodeficiency. Perhaps through merging imaging and therapy in the form of theranostic agents and by avoiding viral vectors, gene transcription-based imaging systems may emerge as a viable option to less specific, low molecular weight molecular imaging agents. Reporter gene transcription-based imaging systems can be applied as therapeutic or theranostic systems simply by employing reporter genes that can serve as therapeutic genes simultaneously, such as NIS and HSV1-TK, or by inserting therapeutic genes in the format of unidirectional or bidirectional vectors. One drawback of using nonviral gene delivery vectors is that the expression of reporter/therapeutic genes is transient since the introduced transgene is not integrated into the host genome. While that makes nonviral vehicles as safer options to viral vectors, the duration of reporter/therapeutic gene expression is only short-lived. Improving the physiological stability of pDNA-nonviral vector complexes can contribute to the prolonged gene expression (Pun and Davis, 2002; Thierry et al., 1997).

In summary, major hurdles to overcome include: (i) lack of strong promoters with universal cancer-specificity; and, (ii) lack of clinically proven gene delivery vehicles. However, we are very optimistic about the potential for the reporter gene transcription-based imaging systems for clinical application, mainly due to the exquisite specificity of the method, which is lacking for most standard molecular imaging agents. We believe rapid advances in nanomaterials will provide us with nonviral gene delivery vehicles with improved safety and efficacy. The vast amount of information accumulated through functional genomics and bioinformatics will enable researchers to discover more accurate and widely applicable cancer-related targets, the promoters of which could be employed for molecular-genetic imaging. Also, recent development of synthetic promoters has important implications for the design of more robust and specific systems.

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We were only able to include a small portion of articles on gene transcription-based cancer imaging in References due to space constraints. We would like to acknowledge all their efforts that have greatly contributed to the field.

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Key facts

- Gene transcription-based imaging or molecular-genetic imaging has been increasingly used as a cancer diagnostic and therapeutic strategy for the last decade.
- Well-characterized reporter gene-imaging probe pairs can be utilized to image cancer of various tissue origins and subtypes.
- The majority of strategies have employed organ- or cell type-specific promoters to drive reporter gene expression in a cancer-specific manner.
- Promoters with universal selectivity for cancer such as the PEG-3 promoter can broaden the application potential of the gene-transcription-based imaging and therapy.

Figure 1. Criteria to consider when developing a reporter transcription-based cancer imaging system

Figure 2. Trends in publication on reporter gene expression-based cancer imaging (and therapy)
(a) The number of publications on gene transcription-based imaging systems each year over the last decade. The Search terms “(cancer or tumor) and specific and promoter and imaging” and “(cancer or tumor) and specific and promoter and imaging and therapy” were used for PubMed database research. Only publications that contain *in vivo* preclinical testing were included. **(b)** Promoters used for cancer imaging in 65 publications shown in **(a)**. (Abbreviations: PSA, prostate-specific antigen; hTERT, human telomerase reverse transcriptase; AFP, α -fetoprotein; CEA, carcinoembryonic antigen; P0, myelin protein zero; hAAT, human α -1-antitrypsin; CCKAR, cholecystokinin type A receptor; ERE, oestrogen-responsive element; HIP, hepatocarcinoma-intestine-pancreas; HRE, hypoxia-responsive element; Id1, inhibitor of DNA binding-1; rNIS, rat sodium iodide symporter; and, PEG-3, progression elevated gene-3).