Topic Introduction

Imaging Mouse Cancer Models In Vivo Using Reporter Transgenes

Scott K. Lyons, P. Stephen Patrick, and Kevin M. Brindle¹

*Department of Molecular Imaging, CRUK Cambridge Research Institute, Li Ka Shing Centre, Cambridge
CB2 0RE, United Kingdom*

Imaging mouse models of cancer with reporter transgenes has become a relatively common experimental approach in the laboratory, which allows noninvasive and longitudinal investigation of diverse aspects of tumor biology in vivo. Our goal here is to outline briefly the principles of the relevant imaging modalities, emphasizing particularly their strengths and weaknesses and what the researcher can expect in a practical sense from each of these techniques. Furthermore, we discuss how relatively subtle modifications in the way reporter transgene expression is regulated in the cell underpin the ability of reporter transgenes as a whole to provide readouts on such varied aspects of tumor biology in vivo.

INTRODUCTION

The past few decades have seen important advances in our ability to image small animals noninvasively in the laboratory. These have been driven largely by the development of preclinical versions of well-established clinical imaging modalities such as MRI (magnetic resonance imaging), PET (positron emission tomography), and ultrasound, as well as other exclusively preclinical modalities such as bioluminescence imaging (BLI). In the preclinical setting, reporter transgenes have proved very useful, in conjunction with several of these small-animal imaging modalities, as a way to generate or enhance gene-expression-dependent image contrast. They provide a means to enhance our ability to detect and monitor populations of labeled cells in vivo, without the need to perform invasive or terminal procedures. In the context of cancer models, reporter transgenes have been instrumental in enabling investigation of diverse aspects of tumor biology noninvasively in vivo. Improvements in our ability to measure dynamically tumor-related parameters, such as growth, metastasis, and protein–protein interactions, have transformed the way that mouse models of cancer can be used for investigations of tumor etiology and also for applied research, such as the development of new cancer therapeutics.

The physical basis of the relevant imaging modalities is discussed elsewhere. The aim of this article is to highlight, in a practical sense, the various ways that reporter transgenes can be used to image mouse models of cancer. The imaging modalities relevant to reporter transgenes will be outlined briefly and the approaches that can be employed to produce a diverse range of reporter-based biological readouts will be discussed.

¹Correspondence: kmb1001@cam.ac.uk

© 2013 Cold Spring Harbor Laboratory Press

Cite this article as *Cold Spring Harb Protoc*; 2013; doi:10.1101/pdb.top069864

TABLE 1. Summary of the practical features of commonly used reporter transgenes for imaging cancer models in vivo

Reporter gene product	Applicable modality	Main advantages	Main disadvantages
Firefly luciferase	Bioluminescence imaging	Rapid, sensitive, relatively low-cost, high-throughput, diverse readouts	Preclinical only, typically 2D image, histological detection challenging
DsRed (<i>Discosoma</i> red fluorescent protein) (or variant)	Fluorescence imaging	Direct visualization of labeled cells on different scales with different hardware, diverse readouts	Depth of penetration (intravital imaging); suboptimal excitation and emission wavelengths, autofluorescent background (whole animal)
Ferritin	Magnetic resonance	Clinical potential, improves contrast of anatomically detailed images	Low throughput, highly technical, relatively low sensitivity, cost
HSV1-tk	Positron emission tomography	Clinical potential, accurate quantitation, enhances image contrast and potentially therapeutic	Radioactive, requires significant infrastructure, cost

WHAT IS A REPORTER TRANSGENE?

Reporter transgenes encode proteins that are capable of enhancing the identification of labeled cells, or of a specific cellular behavior, among a population of unlabeled cells. There are many characteristics of a protein that determine whether it is a good in vivo reporter. These include that its expression confers robust and reporter-specific positive contrast (such that the signal is at least an order of magnitude greater than the noise) and that its expression and function are nontoxic, do not affect normal cellular behavior, and can be quickly detected and quantified at both cellular and macroscopic levels. For certain experimental applications, it might also be desirable that the reporter function as a monomer is not too large (<50 kDa) and is amenable to fusion with other peptides. No reporter transgene currently satisfies all of these criteria—therefore, the reporter and imaging modality need to be chosen on the basis of specific experimental requirements (see Table 1).

A BRIEF OUTLINE OF THE RELEVANT IMAGING MODALITIES

Bioluminescence Imaging

Bioluminescence imaging (BLI) of mammalian cells relies on the detection of light produced by the enzymatic oxidation of an exogenous substrate. A key reason why BLI is considered the most sensitive preclinical imaging modality is because these bioluminescent enzymes do not occur naturally in mammalian cells and, consequently, background bioluminescence emanating from nonlabeled cells is very low. Multiple bioluminescent enzymes, derived from a range of insects and marine creatures, have been described that are capable of expression and light production in mammalian cells. These enzymes can be broadly separated into two substrate classes: those that oxidize luciferin and those that oxidize coelenterazine (see Fig. 1). As these substrates do not cross-react with enzymes of the other class, dual-labeling strategies are possible whereby two biological parameters can be monitored sequentially and noninvasively in vivo. By far the most commonly used bioluminescent enzyme to date is firefly (*Photinus pyralis*) luciferase. This enzyme was one of the first to be used for BLI of mammalian cells in vivo, but has retained its popularity owing to its relatively bright and red-shifted emission and the ease with which its substrate luciferin is handled, administered, biodistributed, and tolerated. Other commonly used bioluminescent enzymes include *Renilla* (*Renilla reniformis*) and more recently *Gaussia* (*Gaussia princeps*) luciferase, the latter being naturally secreted by the cell. Both of these enzymes use coelenterazine as a substrate and do not require ATP to generate bioluminescence. These enzymes therefore permit imaging strategies to measure biological parameters in the

FIGURE 1. Bioluminescent reactions. The reactions catalyzed by the luciferin- and coelenterazine-oxidizing classes of luciferase reporter transgenes. The chemical structures of the luciferase substrates and their oxidized products are indicated, together with the predominant color and wavelength of the emitted light. (Figure adapted from Badr and Tannous 2011.)

extracellular space, which the luciferin-oxidizing enzymes cannot do. Taken in a different biological context, however, the ATP requirement of firefly luciferase can be viewed as highly advantageous, as only viable cells can generate light. Therefore, the cytotoxic activity of new anticancer therapeutics can be assessed within 24 h when using these reporters, which in most cases is significantly faster than observation of gross changes in tumor morphology.

Key Features of BLI

BLI is generally considered the most sensitive *in vivo* preclinical imaging modality currently available, and, when conditions are optimal, it has been shown to be capable of detecting one to three cells residing subcutaneously within a mouse (Rabinovich et al. 2008; Kim et al. 2010). Not all bioluminescence experiments conform to these optimal conditions, however, and the main factors affecting imaging sensitivity are (i) the extent of transgene expression, (ii) the depth of overlying tissue, and (iii) background arising from proximity to other sources of bioluminescence.

Addressing the first point is simple: As luciferase expression is in most cases well tolerated, one can use cells with high levels of expression. For the second point, overlying tissue is not optically transparent and the wavelengths of light generated by common luciferase reporters (460 nm to 580 nm) are prone to absorption and scatter by this tissue, such that imaging sensitivity drops approximately 10-fold per centimeter of tissue depth. Fortunately, this is about the limit of tissue depth for any mouse model as, any deeper, and the mouse can simply be turned over and imaged from a different aspect. Highly pigmented organs, blood, and dark fur also absorb light and can significantly compromise imaging sensitivity.

The third point is relevant when attempting to detect metastases *in vivo* and the primary luciferase-expressing tumor has developed to a large size and produces a lot of light. If the primary tumor is distant from the site of metastasis development, physical shielding of the primary tumor from the camera can aid visualization of small metastases. Shielding is not possible for metastases close to the primary tumor; however, it might be possible to improve metastasis detection by surgically removing the primary tumor. Alternatively, metastatic frequencies can be determined at the end of an experiment as isolated organs retain bioluminescence and can be imaged for up to 30 min *ex vivo*. This third point is also a relevant concern when attempting to image tumors arising in genetically engi-

neered mouse (GEM) models, where luciferase is expressed by a large number of nontransformed cells, as will be discussed later.

BLI is also a rapid imaging modality. Up to five mice can be imaged simultaneously and a typical scan takes less than a minute to acquire (ranging from 1 to 300 sec, depending upon the level of signal). As mentioned later, BLI is also highly versatile, and many different aspects of tumor biology can be investigated, depending upon how expression of the luciferase enzyme itself is regulated.

Immediately before imaging, the animal is injected with a bioluminescent substrate. Luciferin-oxidizing enzymes (e.g., firefly luciferase) exhibit relatively slow “glow” kinetics and the substrate can be injected intraperitoneally (i.p.). Biodistribution is relatively rapid, and, depending on the location of labeled cells in the body, peak levels of bioluminescence are usually reached 10–14 min after injection. The intensity of light emission then falls slowly, to reach undetectable levels within ~2 h. Coelenterazine-oxidizing enzymes exhibit relatively fast “flash” bioluminescence kinetics, and intravenous (i.v.) injection of the substrate is preferred to achieve faster biodistribution (even though i.v. administration likely limits the total number of imaging sessions per experimental subject). Coelenterazine can also be injected i.p., but, as this substrate is prone to auto-oxidation, i.p. injections can give rise to the appearance of a bioluminescent “pool” of low-intensity background light emanating from the abdomen of the mouse irrespective of whether labeled cells are present.

Fluorescence Imaging

Conventional fluorescence imaging of a dye, probe, or reporter transgene product all rely on the following principal—that the fluorescent material is excited by light energy of a specific wavelength, which in turn is then released in the form of light with a longer wavelength and also heat (by contrast, multiphoton microscopy utilizes longer-wavelength excitation to produce shorter-wavelength emission plus heat). It is the emitted longer-wavelength light or fluorescence that is detected in conventional fluorescence imaging.

In terms of fluorescent reporter transgenes, a broad palette of colors has been made available to the research community (Shaner et al. 2004), making it theoretically possible to monitor multiple biological parameters in the same cell. These reporters can simply be used to label a defined cell type or biological parameter or they can be fused to other peptides of interest to measure specific protein–protein interactions or localization within the cell (as discussed later).

Two main fluorescence-based techniques have been developed that are suitable for in vivo imaging with reporter transgenes—one on the subcellular scale termed intravital microscopy and the other a macroscopic whole-animal imaging technique (Fig. 2). A significant advantage of fluorescence imaging over other in vivo imaging approaches is the ability to generate sufficient signal to visualize single cells directly with subcellular resolution. Intravital microscopy, achieved with multi-photon excitation (Entenberg et al. 2011) or by spinning disc confocal microscopy (Egeblad et al. 2008), has revolutionized our ability to visualize cellular behavior directly in vivo. The technique allows direct visualization of the interactions of distinct cell populations, each labeled with a unique fluorescent color, within the tumor microenvironment. This approach has proved extremely valuable for direct visualization of the early extravasation stages of metastasis, as well as tumor cell interactions with the microenvironment and the behavior of immune cells within the tumor (Kedrin et al. 2008; Giampieri et al. 2009; Egeblad et al. 2010; Nakasone et al. 2012).

Clearly, direct visualization of tumor cell behavior in vivo is a powerful means for advancing our understanding of tumor biology. A downside, however, is the depth of penetration of this technique, which is <0.5 mm. This limits intravital imaging to studies of the skin or necessitates the use of window chambers for deeper tissues with associated shorter-term experimental goals. The field of view associated with this technique is also very small compared with those of other whole-animal imaging techniques discussed here, and it is challenging to maintain the same field of view when multiple imaging time points are desired (photoactivatable reporters have been useful in this regard [Chudakov et al. 2007]).

FIGURE 2. Typical optical reporter-transgene images. (A) Longitudinal BLI study with an orthotopic brain tumor model. The *top* row of images shows an untreated control followed for 4 wk, versus the *bottom* row, which shows a mouse treated with the alkylating agent temozolomide, which initially responds to treatment (days 29–34), but subsequently relapses (Hashizume et al. 2010). (B) Longitudinal Intravital imaging showing the development of a red fluorescent protein (RFP)-labeled metastasis in the brain (Kienast et al. 2010). (C) Conventional whole-animal fluorescence image showing Kat2 (which is an RFP)-labeled A-431 tumor cells that express high levels of epidermal growth factor receptor (EGFR), growing subcutaneously in the mouse (Pardo et al. 2012). (Images reproduced by kind permission of the publishers.)

In terms of whole-animal fluorescence imaging, using either epi- or trans-illumination, fluorescent reporter transgenes have to date had relatively limited utility and have been better suited to imaging superficial and well-established tumors with very high reporter expression levels (Yang et al. 2009). This is primarily due to the excitation and emission wavelengths of light associated with fluorescent reporter transgenes. As shown in Figure 3, the wavelengths of light in the visible spectrum are prone to absorption by overlying tissues, with red-shifted wavelengths performing better than those associated with the ubiquitous green fluorescent protein (GFP) reporter. Moreover, nonlabeled structures found abundantly in the body (e.g., collagen in the extracellular matrix) can generate considerable amounts of autofluorescence when these excitation wavelengths are used. Thus, even though fluorescence imaging generates significantly more light than a bioluminescence imaging experiment, the overall reduction in signal to background limits the sensitivity of the whole-animal imaging experiment. Sophisticated image-analysis methods, such as spectral unmixing (Xu and Rice 2009) and dynamic contrast enhancement (DyCE) (Hillman and Moore 2007; Hillman et al. 2011), can be used to enhance the fluorescence signal over background. Such techniques can help to deconvolute signals arising from multiple labels, should more than one biological parameter be measured.

An important advance for whole-animal fluorescence imaging came recently with the development of reporters suitable for *in vivo* imaging that fluoresce in the near-infrared region of the electromagnetic spectrum (Shu et al. 2009; Filonov et al. 2011). One is an engineered (monomeric) form of a bacteriophytochrome from *Deinococcus radiodurans* called IFP1.4 (excitation 684 nm, emission 708 nm). The other is a molecular dimer named iRFP (excitation 690 nm, emission 713 nm), developed by random mutagenesis of a bacteriophytochrome from *Rhodospseudomonas palustris*. Both reporters spontaneously incorporate biliverdin, an endogenously produced heme breakdown

FIGURE 3. The optical window. The efficiency of light transmission through tissue (Hb, deoxyhemoglobin; HbO₂, oxyhemoglobin). The visible portion of the electromagnetic spectrum has been highlighted, along with the wavelengths commonly used by optical reporters, illustrating why red-shifted and near infra-red (NIR) wavelengths of light are preferable for optical imaging in vivo.

product, as the chromophore to generate fluorescence. Although iRFP was shown to be effectively brighter than IFP1.4 in vivo, it should be noted that both reporter transgenes are relatively dim when compared with the commonly used fluorescent reporter transgenes. Undoubtedly, their further development holds great promise for in vivo imaging, as their excitation and emission wavelengths are better suited for transmission through tissue and, with the appropriate hardware, should facilitate imaging on both micro- and macroscopic scales.

Magnetic Resonance Imaging

Magnetic resonance Imaging (MRI), as most commonly used, creates image contrast based on the spin-relaxation behavior of the ¹H nuclei of tissue water, with contrast being affected by tissue composition, water content and the presence of paramagnetic species. Other nuclei, such as ¹³C, ¹⁹F, and ³¹P, can also be used with MRI, but, as the majority of the body consists of water, ¹H imaging is much more sensitive and can give anatomical detail at relatively high resolution (in preclinical studies, isotropic image resolutions of 30 μm are possible [Boretius et al. 2009]). As the physical concepts are quite complex, a comprehensive introduction to MRI is beyond the scope of this article. However, a very readable account can be found in Gadian's *NMR and Its Applications to Living Systems* (Gadian 1995).

In comparison with optical imaging, the equipment required for MRI is relatively large and expensive to operate. MRI is also a low-throughput technique (typically 5–60 min per scan per individual) and requires more training before operation of the equipment is possible. However, MRI also has some significant advantages and enables imaging of deep organs and tumors in vivo in three dimensions. Furthermore, the use of specialized imaging sequences, and also the detection of other nuclei (e.g., ³¹P, ¹³C, ¹⁵N, ²³Na) as well as ¹H, can reveal a great deal of information about a variety of biological processes, including brain function and damage, blood flow and oxygenation,

FIGURE 4. Positron emission tomography (PET) and magnetic resonance reporter transgene images. (A) PET images of a subcutaneous LAPC-4 (prostate) xenograft tumor labeled with *HSV1-tk*, before and after treatment with ganciclovir (Min and Gambhir 2008). (B) MRI images of a C6 glioma tumor and the change in contrast mediated by the induction (Tet-on) of ferritin expression (Cohen et al. 2005). (Images reproduced by kind permission of the publisher.)

hemorrhage, tumor pH, and metabolism. It also has an advantage over several other whole-body noninvasive imaging modalities (PET; single-photon emission computed tomography [SPECT]; and computed tomography [CT]) in that ionizing radiation is neither required nor generated.

Magnetic Resonance Reporters

Despite the fact that a large number of magnetic resonance (MR) reporters have been developed and described in the literature, few have ever been used to answer real biological questions. Their absence in mainstream molecular biology likely reflects the difficulty of developing effective MR reporters (Gilad et al. 2007b) and the relative complexity of the modality. Ferritin, however, has been used in considerably more studies than other published MR reporters (Cohen et al. 2005; Genove et al. 2005; Iordanova and Ahrens 2012; Vande Velde et al. 2012). Ferritin is a hollow spherical polymeric protein that accumulates and stores a nontoxic mineral form of iron in its internal cavity (Fig. 4). This iron core is partially superparamagnetic, and, when present in tissue, produces negative contrast (a hypointense signal) in T_2 -weighted ^1H MR images. The advantages of this system are that a relatively large amount of contrast can be produced in comparison with other MR reporters and that endogenous iron can be used as the substrate. Disadvantages include that the contrast produced is negative (that the “signal” produced is actually an absence of signal) and that temporal resolution is low because the buildup of ferritin within the cell is rate-limited by iron availability.

Tumor models have been a popular choice for proof-of-concept studies for novel MR reporters as they provide a large imageable mass in vivo and as cancer cells are in general easy to grow and genetically modify. Perhaps there has been a resulting tendency to assume that MR reporters could be of use in answering relevant questions about tumor biology (Hasegawa et al. 2010), but this is not necessarily the case. For example, the utility of ferritin, or indeed any of the several iron-based MR reporter systems (Weissleder et al. 1997, 2000; Deans et al. 2006; Zurkiya et al. 2008; Cui et al. 2010) with regard to cancer, might be limited by the fact that a perturbed iron metabolism is a feature of many tumor types; iron-import proteins are up-regulated and iron-storage proteins (ferritin) down-regulated (Gatter et al. 1983; Hogemann-Savellano et al. 2003; Herbison et al. 2009; Okazaki et al. 2010). These changes, which correlate with tumor grade (Prutki et al. 2006; Zhang et al. 2008), increase the bioavailable iron pool available for the needs of the proliferating cell. As we have mentioned, an important property of a good reporter transgene is that it does not alter the system under observation (“theragnostics” aside). Use of an iron-based reporter in a tumor model might therefore have unpredictable effects, such as increasing proliferation or the generation of reactive oxygen species via the Fenton reaction (Toyokuni 2009), thus potentially confounding the information provided by the reporter.

Alternatives and the Future of MR Reporters

Many published MR reporters (e.g., those used in conjunction with T_1 -weighted imaging and chemical exchange saturation transfer [CEST], and non- ^1H -based MR spectroscopy or MRI), however, do

not rely on iron for the production of contrast and also have certain properties that might favor their use, were it not for the low sensitivity of detection or low signal-to-background ratios from which they generally suffer.

For example, lysine-rich protein (LRP), a reporter gene product for CEST, allows contrast to be turned on and off (Gilad et al. 2007a) and also the use of multiple “colors.” Positive contrast (a hyperintense signal) can be produced by β -galactosidase in T_1 -weighted imaging (Louie et al. 2000), which in many cases is preferable to the generation of negative contrast. Furthermore, hyperpolarized MRI has already demonstrated its value in the study of cancer (Day et al. 2007; Gallagher et al. 2008) and offers a potential way to increase the MR signal radically, but thus far potential reporters have only been demonstrated in vitro (Jamin et al. 2009; Chen et al. 2011).

Positron Emission Tomography

PET is a radionuclide-based imaging technique and is currently the most sensitive clinical imaging modality available. PET imaging (and microPET, its small-animal equivalent) relies upon the detection of trace amounts of a molecular probe (e.g., glucose or acetate) that has been labeled with a positron-emitting isotope (e.g., ^{18}F or ^{11}C). The emitted positron (β^+ , the antimatter equivalent of an electron) itself is not detected. Instead, the emitted positron travels a short distance through tissue before it encounters an electron. The resulting annihilation event produces two 511-keV γ rays that travel in opposite directions relative to one another. It is the coincident detection of these γ rays by a ring of detectors that enables the location of the labeled probe to be calculated.

PET imaging can be advantageous as, unlike optical imaging, its signal is barely impeded by overlying tissue (attenuation can be mapped using CT [Kinahan et al. 2003]). Thus the location of picomolar amounts of probe can be readily detected and accurately quantified irrespective of tissue depth (Muzic et al. 2000; Madsen et al. 2011). As the positron travels a short distance before annihilation, the resolution of this technique is $\sim 1\text{ mm}^3$ and produces images showing areas of relative signal intensity as opposed to anatomic detail. The half-life of the commonly used positron emitters is relatively short (e.g., ^{18}F , 110 min; ^{11}C , 20 min), and therefore the total exposure to ionizing radiation per scan is relatively low and should have limited influence on the biology of the model. As every positron, irrespective of the emitting isotope, produces γ rays of equivalent energy upon annihilation, PET is a “single-color” imaging modality, and the signals arising from multiple probes cannot be differentiated on the same scan. A further disadvantage of PET imaging is that it is radioactive and the handling of probes and imaging subjects is subject to much stricter regulation than that for nonradioactive imaging approaches. The production of labeled probes can also be expensive or necessitate proximity to a cyclotron. Routine microPET scans range from 10 to 60 min, depending upon whether static or dynamic measurements are being made, and multiple subjects can be imaged at once, subject to an appropriate imaging module (Aide et al. 2012).

Several PET reporter transgenes have been developed for use in conjunction with preclinical models of cancer. Thymidine kinase derived from herpes simplex virus (*HSV1-tk*) is probably the best known of these (Fig. 4) and has even been used to a limited extent in the clinic. This enzyme preferentially phosphorylates radiolabeled nucleoside probes (^{18}F -labeled FIAU, FMAU, FEAU, FHGB), trapping them in the cell. It is the preferential accumulation of these probes in reporter-expressing cells that generates image contrast. Several point mutants, including *HSV1-ser39tk*, *HSV1-tk(A168H)*, and a double-mutant *HSV1-A167Ysr39tk* (Likar et al. 2008), have been developed with enhanced imaging properties (Gambhir et al. 2000; Likar et al. 2008; Najjar et al. 2009). *HSV1-tk* will also preferentially activate the prodrug ganciclovir over endogenous thymidine kinase, making it potentially a therapeutic transgene as well as an imaging one.

Other notable PET reporter transgenes include the sodium iodide symporter (NIS), which mediates the import of the radiolabeled probe into the cell (^{124}I for low-resolution PET, $^{99\text{m}}\text{TcO}_4$ -pertechnetate for SPECT imaging and ^{131}I for therapy [Groot-Wassink et al. 2004; Schipper et al. 2007; Liu et al. 2012]) and the plasma membrane somatostatin receptor (2SSTR2), which binds

radiolabeled analogs of somatostatin (^{68}Ga for PET, ^{111}In for SPECT and ^{90}Y for therapy [Nicolas et al. 2011; Singh et al. 2011; Zhang et al. 2011]).

REPORTER-TRANSGENE-BASED IMAGING IS HIGHLY VERSATILE

Over the course of research spanning several decades, many methods have been developed to regulate transgene expression in the mouse. Accordingly, these methods have been utilized to generate imaging transgenes that report noninvasively upon diverse aspects of tumor biology in vivo.

Constitutive Expression Can Be Used to Measure Tumor Burden

The vast majority of imaging performed in vivo with a reporter transgene utilizes a straightforward labeling approach whereby the reporter transgene is simply expressed at a constitutive, usually high, level in the cell type or tissue of choice. Typically, this approach enables noninvasive monitoring of the relative number and location of labeled cells in vivo. In the context of cancer models, this approach has proven very useful for following the dynamics of tumor growth and metastasis in vivo (Goldie et al. 2012), for monitoring tumor response to treatment (Jenkins et al. 2003; Zeng et al. 2010), and for studying the effects of adding or subtracting cancer-related gene expression on tumor biology (e.g., oncogene-addiction studies [Rakhra et al. 2010]).

For xenograft-based tumor models, or primary tissue explant models, this labeling strategy can be achieved practically by simply transfecting or transducing cells in vitro with a vector that uses a constitutively expressed promoter to drive expression of the reporter transgene. Viral promoters, such as CMV (cytomegalovirus) or SV40 (simian virus 40), are capable of driving high levels of expression in a broad variety of mammalian cell types—but can be prone to silencing (Qin et al. 2010). Eukaryotic constitutive promoters therefore can be preferable (e.g., EF1, CAGGS [part CMV, part chicken β -actin] for high levels of expression, or UBC or PGK for lower levels of expression). Polyclonal populations of labeled cells can be generated relatively quickly in culture, but can exhibit more heterogeneous levels of reporter transgene expression relative to pure clones. This variability can be a confounding issue for certain experimental applications such as cancer stem cell or metastasis tracking studies. Alternatively, pure clones of reporter-expressing cells can be derived, but this typically necessitates a longer period in culture, and the process of cloning itself can result in the expansion of rare variant cells that are poorly representative of the original parental population. Parameters such as proliferation rate and possibly the response to a control therapeutic should be assessed before the in vivo application of cloned cells.

Spontaneous tumors in GEM models can also be imaged with a constitutively expressed reporter to determine relative cell number. First, however, a reporter expression cassette must be stably introduced into the transgenic model. Conventionally, this has been achieved through the germline. This process can be time-consuming even if an appropriate transgenic reporter mouse strain already exists, particularly if the tumor genotype of the GEM comprises multiple segregating alleles. If no reporter strains exist, new transgenic strains can be generated and characterized in ~12–18 mo.

A key consideration with this strategy is to ensure that reporter expression is restricted as much as possible to tumor cells and not to nontransformed cells within the vicinity. Achieving this greatly improves imaging signal-to-background values, thereby improving sensitivity and decreasing the lag time before tumor development can be detected unambiguously and monitored. Several tissue-specific promoters have worked well in this regard as their native expression is tightly restricted to small organs (e.g., pituitary and prostate [Vooijs et al. 2002; Lyons et al. 2006]). This approach is poorly suited for imaging tumors that arise from relatively large organs in the mouse (e.g., liver), as baseline light emission originating from a large number of normal nontransformed cells will greatly reduce sensitivity and the ability to detect anything other than the largest and most advanced tumors.

Several laboratories have developed Cre-dependent reporters as an alternative way to achieve tumor-cell-specific reporter expression in GEM models (Lyons et al. 2003; Safran et al. 2003). Such

mice possess a silent reporter transgene that has the ability to “switch on” and express in any cell of the body following Cre-mediated recombination. Once bred onto an appropriate tumor-initiating genetic background, tissue-specific Cre lines can be used to initiate imageable tumorigenesis. Similar to tissue-specific reporters, broad Cre (and consequently reporter) expression in large organs will seriously compromise imaging sensitivity. Consequently, conditional reporter strains are likely to be most useful in the context of GEM models that rely upon adenoviral or lentiviral delivery of Cre recombinase to initiate tumor development (Kirsch et al. 2007; DuPage et al. 2009). Reporter expression (and tumor-initiated cells) can be limited to infected and sporadically recombined cells within the desired tissue rather than expressed uniformly throughout.

A further highly innovative Cre-dependent reporter mouse strain, termed “R26R-confetti” (Snipert et al. 2010) (based on the “brainbow 2.1” reporter [Livet et al. 2007]) relies upon Cre to switch randomly a tandem array of floxed red, yellow, green, and blue reporters to generate one of four colors. This approach is theoretically compatible with intravital microscopy (as discussed above in the fluorescence imaging section) and recently provided direct evidence for the existence of cancer stem cells within adenomas of the intestine (Schepers et al. 2012). A similar stochastic approach, this time relying on the random transduction of three independent lentiviruses containing either a red, green, or blue fluorescent protein (termed “RGB imaging”), has also been used to determine tumor clonality and cell lineage tracing of both primary and metastatic lesions (Weber et al. 2011).

An alternative, ES-cell-based chimera approach has been described recently that greatly reduces the breeding requirements associated with the development of imageable GEMs (Heyer et al. 2010). This approach is attractive as, with the advent of defined media conditions (Nichols et al. 2009), it is now relatively straightforward to generate and maintain ES cells from preexisting GEM strains. These cells therefore already possess a baseline tumor-initiating genotype and, as ES cells are also easy to manipulate *in vitro*, the expression of a reporter transgene can be added or the expression of further tumor-related genes can be manipulated before the generation of chimeras (Zhou et al. 2010). Factors likely to influence the imaging sensitivity of the resultant reporter-chimeric mice will be similar to germline reporter GEMs, especially if the contribution of modified ES cells is high in the chimera. A lower ES cell contribution in the chimera therefore might be desirable in terms of improving imaging sensitivity, but might also extend tumor latency or reduce tumor penetrance. (Note too that impressive results [i.e., very low levels of background bioluminescence from nonrecombined tissue and good levels of light output following Cre-mediated recombination] have also been seen with an unpublished line developed by the Jacks laboratory and available from the NCI mouse repository [strain number 01XAC, common strain name: ROSA26-pCAGGs-LSL-luciferase, strain nomenclature: B6.129-Gt[ROSA]26Sor^{tm2(ACTB-Luc)Tyj}].)

Reporters can also be regulated by promoters possessing activities that are dependent on biological context. For example, a reporter transgene driven by the S-phase-specific E2F promoter was used to show that the mechanism of a drug was cytostatic rather than cytotoxic in a mouse model of glioma (Uhrbom et al. 2004). Cellular responses to genotoxic stress have also been imaged *in vivo* using both p53-responsive (Wang and El-Deiry 2003) and p21 promoters (Tinkum et al. 2011). Another promoter, called PEG3, has intriguingly been shown to possess tumor-specific activity (Bhang et al. 2011). When a PEG3-regulated transgene was administered systemically to a xenograft model of lung cancer, enhanced reporter expression was observed from tumors relative to surrounding tissue and control vectors.

Coexpression and Reporter Tagging Strategies

In some circumstances, it can be desirable to use the readout of a reporter transgene to indicate the coexpression of a second, tumor-biology-related, gene. In general, this is best achieved by co-introducing the expression of both the reporter transgene and the gene of interest in the same vector and driven by the same promoter. This can be achieved in multiple ways. A fusion protein can be generated, if the gene of interest will functionally tolerate a peptide tag, by cloning both genes *cis*, in-frame and removing the stop codon of the upstream gene. Most monomeric fluorescent

and bioluminescent reporters will retain function with a carboxy- or amino-terminal tag, but it should be noted that not all genes of interest will retain full functionality with the addition of a large peptide domain (e.g., mStrawberry is 28 kDa). The functionality of some fusion proteins can be improved by positioning a peptide linker (e.g., polyglycine) between the genes.

Alternatively, both genes can be coexpressed from the same promoter using an internal ribosome entry sequence (IRES) (Mokrejs et al. 2006), although it has been noted that the relative expression levels of both genes are not necessarily equal and can differ markedly (Mizuguchi et al. 2000). This means that the extent of reporter transgene expression is not necessarily indicative of the expression levels of the gene of interest.

A further, relatively recent, coexpression strategy is to position a 2A sequence in-frame between the reporter and gene of interest (Szymczak et al. 2004). Like the construction of a fusion transgene, it is essential to remove all but the final stop codon. 2A sequences originate from picornaviruses and are relatively short (~20 amino acids) peptide sequences. During protein translation, a peptide bond fails to form between amino acids coded by the 2A coding sequence, but crucially translation of the mRNA continues. This results in separate and equimolar expression of both the reporter and gene of interest. This is advantageous as the relative expression level of the gene of interest can now be estimated on the basis of reporter-transgene expression levels. It should also be noted, however, that the upstream gene(s) in a 2A sequence will retain the 2A amino acids tagged to its carboxyl terminus. Again, most monomeric reporters will tolerate such a tag, whereas this might not always be the case for all genes of interest.

Using a mixture of these coexpression strategies, several laboratories have developed double and triple reporter-transgene cassettes (Kesarwala et al. 2006; Ray et al. 2007). The general idea is to generate a single expression construct that confers the ability (and the inherent advantages therein) to image labeled cells with a variety of modalities. For example, a triple reporter comprising luciferase, *HSV1-tk* and GFP permits rapid and sensitive in vivo BLI, tomographic radionuclide imaging with accurate 3D image reconstruction (PET), as well as microscopy, flow cytometry, and histological identification of cells ex vivo (fluorescence) at the end of an experiment.

Short-hairpin RNAs can also be coexpressed with reporter transgenes (Premssirut et al. 2011; Zuber et al. 2011). Vectors have been developed whereby a micro-RNA (miR) sequence containing the short-hairpin RNA (shRNA) is positioned immediately after the stop codon of a reporter. Again, the extent of reporter-transgene expression should be representative of miR expression levels, thus providing an indirect method to visualize gene knockdowns.

Imaging Other Biological Parameters

The expression of a transgene can also be regulated at the posttranslational level, and peptide motifs conferring specific biological properties can be fused to a reporter transgene to convey unique functionality. For example, a cell-cycle-specific reporter system, termed fluorescence ubiquitination cell cycle indicator (FUCCI), has been developed by fusing peptide domains from Cdt1 and geminin to both a red and a green fluorescent protein, respectively (Sakaue-Sawano et al. 2008). Both reporters are constitutively expressed, but only one color (protein) is stable at any point in the cell cycle, making the cell appear either red or green, depending upon whether the cell is resting (G_1) or dividing ($S-G_2-M$). This approach has been well validated in the context of xenografted tumors, although it has been noted that FUCCI-labeled cells treated with microtubule inhibitors can result in a counterindicative readout (Kaida et al. 2011), possibly owing to an essential role played by Cdt1 at M phase (Varma et al. 2012).

Some genetically encoded reporters are also amenable to split-reporter strategies. That is, the reporter can be split into two inactive halves, which in turn are then fused to two independent peptide domains. Should those peptide domains come into close proximity, through binding, then the two reporter halves come together, partially restoring reporter function. Firefly (Luker et al. 2002), *Renilla* (Paulmurugan and Gambhir 2003), and *Gaussia* luciferases (Kim et al. 2009), as well as *HSV1-tk* (Massoud et al. 2010), have all been split in this way. The tumor biology that these reporters have been able to report on in vivo includes c-Myc activation (Fan-Minogue et al. 2010), Her2 pathway

activation (Wolf et al. 2011) and the assembly of DNA double-strand-break repair complexes (Li et al. 2011).

Split reporters physically connected by a “smart” peptide linker have also been developed and have been used to measure parameters such as apoptotic cell death and promitogenic signaling. The apoptosis reporter is constitutively inactive and relies upon caspase-3-mediated cleavage of the peptide linker to free both inactive reporter halves, which subsequently bind together, reconstituting reporter function (Coppola et al. 2008). Another reporter utilizes a peptide linker that alters its conformation based upon Akt-mediated phosphorylation (Zhang et al. 2007). When Akt (protein kinase B) signaling is active, the linker is phosphorylated and assumes a shape that prevents the reconstitution of reporter function. When Akt signalling is chemically inhibited, however, the nonphosphorylated linker changes conformation, bringing both reporter halves into close proximity and producing light.

CONCLUDING REMARKS

Undoubtedly, the application of reporter transgenes in vivo has transformed the way that mouse models of cancer can be utilized in the laboratory. Many aspects of tumor biology, including response to treatment, can now be visualized noninvasively in the same subject, thus greatly improving the quality of the in vivo data while simultaneously reducing the number of animals needed to test a hypothesis.

In terms of the future of reporter transgenes and cancer models, new reporters with unique properties continue to be developed (near infra-red [NIR] fluorescent reporters, positive-contrast MR reporters, etc.), and the innovative ways that their expression can be regulated continues to produce ever-more-diverse biological readouts. New imaging modalities are also being developed that will be able to image new classes of reporter. For example, photoacoustic (optoacoustic) imaging (de la Zerda et al. 2012; Razansky et al. 2012), which combines the sensitivity and spectral resolution of optical imaging with the spatial resolution of ultrasound imaging, will theoretically show enhanced contrast with genetically encoded molecules that can efficiently absorb light and convert that energy into heat.

Taken together, the preclinical application of reporter transgenes in conjunction with mouse models of cancer continues to enlighten and inform and to make a significant, albeit indirect, impact on the clinical management of the disease.

ACKNOWLEDGMENTS

The authors’ research is primarily funded by Cancer Research UK. We would also like to acknowledge the contributions made by other researchers that could not be covered or cited in the text owing to space restrictions.

REFERENCES

- Aide N, Visser EP, Lheureux S, Heutte N, Szanda I, Hicks RJ. 2012. The motivations and methodology for high-throughput PET imaging of small animals in cancer research. *Eur J Nucl Med Mol Imaging* 39: 1497–1509.
- Badr CE, Tannous BA. 2011. Bioluminescence imaging: Progress and applications. *Trends Biotechnol* 29: 624–633.
- Bhang HE, Gabrielson KL, Laterra J, Fisher PB, Pomper MG. 2011. Tumor-specific imaging through progression elevated gene-3 promoter-driven gene expression. *Nat Med* 17: 123–129.
- Boretius S, Kasper L, Tammer R, Michaelis T, Frahm J. 2009. MRI of cellular layers in mouse brain in vivo. *NeuroImage* 47: 1252–1260.
- Chen AP, Hurd RE, Gu YP, Wilson DM, Cunningham CH. 2011. ¹³C MR reporter probe system using dynamic nuclear polarization. *NMR Biomed* 24: 514–520.
- Chudakov DM, Lukyanov S, Lukyanov KA. 2007. Tracking intracellular protein movements using photoswitchable fluorescent proteins PS-CFP2 and Dendra2. *Nat Protoc* 2: 2024–2032.
- Cohen B, Dafni H, Meir G, Harmelin A, Neeman M. 2005. Ferritin as an endogenous MRI reporter for noninvasive imaging of gene expression in C6 glioma tumors. *Neoplasia* 7: 109–117.
- Coppola JM, Ross BD, Rehemtulla A. 2008. Noninvasive imaging of apoptosis and its application in cancer therapeutics. *Clin Cancer Res* 14: 2492–2501.
- Cui W, Liu L, Kodibagkar VD, Mason RP. 2010. S-Gal, a novel 1H MRI reporter for β -galactosidase. *Magn Reson Med* 64: 65–71.
- Day SE, Kettunen MI, Gallagher FA, Hu DE, Lerche M, Wolber J, Golman K, Ardenkjaer-Larsen JH, Brindle KM. 2007. Detecting tumor response to treatment using hyperpolarized ¹³C magnetic resonance imaging and spectroscopy. *Nat Med* 13: 1382–1387.

- Deans AE, Wadghiri YZ, Bernas LM, Yu X, Rutt BK, Turnbull DH. 2006. Cellular MRI contrast via coexpression of transferrin receptor and ferritin. *Magn Reson Med* 56: 51–59.
- de la Zerda A, Bodapati S, Teed R, May SY, Tabakman SM, Liu Z, Khuri-Yakub BT, Chen X, Dai H, Gambhir SS. 2012. Family of enhanced photoacoustic imaging agents for high-sensitivity and multiplexing studies in living mice. *ACS Nano* 6: 4694–4701.
- DuPage M, Dooley AL, Jacks T. 2009. Conditional mouse lung cancer models using adenoviral or lentiviral delivery of Cre recombinase. *Nat Protoc* 4: 1064–1072.
- Egeblad M, Ewald AJ, Askautrud HA, Truitt ML, Welm BE, Bainbridge E, Peeters G, Krummel MF, Werb Z. 2008. Visualizing stromal cell dynamics in different tumor microenvironments by spinning disk confocal microscopy. *Dis Model Mech* 1: 155–167; discussion 165.
- Egeblad M, Nakasone ES, Werb Z. 2010. Tumors as organs: Complex tissues that interface with the entire organism. *Dev Cell* 18: 884–901.
- Entenberg D, Wyckoff J, Gligorijevic B, Roussos ET, Verkhusha VV, Pollard JW, Condeelis J. 2011. Setup and use of a two-laser multiphoton microscope for multichannel intravital fluorescence imaging. *Nat Protoc* 6: 1500–1520.
- Fan-Minogue H, Cao Z, Paulmurugan R, Chan CT, Massoud TF, Felsher DW, Gambhir SS. 2010. Noninvasive molecular imaging of c-Myc activation in living mice. *Proc Natl Acad Sci* 107: 15892–15897.
- Filonov GS, Piatkevich KD, Ting LM, Zhang J, Kim K, Verkhusha VV. 2011. Bright and stable near-infrared fluorescent protein for in vivo imaging. *Nat Biotechnol* 29: 757–761.
- Gadian DG. 1995. *NMR and its applications to living systems*. Oxford University Press, Oxford.
- Gallagher FA, Kettunen MI, Day SE, Hu DE, Ardenkjaer-Larsen JH, Zandt R, Jensen PR, Karlsson M, Golman K, Lerche MH, et al. 2008. Magnetic resonance imaging of pH in vivo using hyperpolarized ¹³C-labelled bicarbonate. *Nature* 453: 940–943.
- Gambhir SS, Bauer E, Black ME, Liang Q, Kokoris MS, Barrio JR, Iyer M, Namavari M, Phelps ME, Herschman HR. 2000. A mutant herpes simplex virus type 1 thymidine kinase reporter gene shows improved sensitivity for imaging reporter gene expression with positron emission tomography. *Proc Natl Acad Sci* 97: 2785–2790.
- Gatter KC, Brown G, Trowbridge IS, Woolston RE, Mason DY. 1983. Transferrin receptors in human tissues: Their distribution and possible clinical relevance. *J Clin Pathol* 36: 539–545.
- Genove G, DeMarco U, Xu H, Goins WF, Ahrens ET. 2005. A new transgene reporter for in vivo magnetic resonance imaging. *Nat Med* 11: 450–454.
- Giampieri S, Manning C, Hooper S, Jones L, Hill CS, Sahai E. 2009. Localized and reversible TGFβ signalling switches breast cancer cells from cohesive to single cell motility. *Nat Cell Biol* 11: 1287–1296.
- Gilad AA, McMahon MT, Walczak P, Winnard PT Jr, Raman V, van Laarhoven HW, Skoglund CM, Bulte JW, van Zijl PC. 2007a. Artificial reporter gene providing MRI contrast based on proton exchange. *Nat Biotechnol* 25: 217–219.
- Gilad AA, Winnard PT Jr, van Zijl PC, Bulte JW. 2007b. Developing MR reporter genes: Promises and pitfalls. *NMR Biomed* 20: 275–290.
- Goldie SJ, Mulder KW, Tan DW, Lyons SK, Sims AH, Watt FM. 2012. FRMD4A upregulation in human squamous cell carcinoma promotes tumor growth and metastasis and is associated with poor prognosis. *Cancer Res* 72: 3424–3436.
- Groot-Wassink T, Aboagye EO, Wang Y, Lemoine NR, Reader AJ, Vassaux G. 2004. Quantitative imaging of Na/I symporter transgene expression using positron emission tomography in the living animal. *Mol Ther* 9: 436–442.
- Hasegawa S, Furukawa T, Saga T. 2010. Molecular MR imaging of cancer gene therapy: Ferritin transgene reporter takes the stage. *Magn Reson Med* 9: 37–47.
- Hashizume R, Ozawa T, Dinca EB, Banerjee A, Prados MD, James CD, Gupta N. 2010. A human brainstem glioma xenograft model enabled for bioluminescence imaging. *J Neurooncol* 96: 151–159.
- Herbison CE, Thorstensen K, Chua AC, Graham RM, Leedman P, Olynyk JK, Trinder D. 2009. The role of transferrin receptor 1 and 2 in transferrin-bound iron uptake in human hepatoma cells. *Am J Physiol Cell Physiol* 297: C1567–C1575.
- Heyer J, Kwong LN, Lowe SW, Chin L. 2010. Non-germline genetically engineered mouse models for translational cancer research. *Nat Rev Cancer* 10: 470–480.
- Hillman EM, Moore A. 2007. All-optical anatomical co-registration for molecular imaging of small animals using dynamic contrast. *Nat Photon* 1: 526–530.
- Hillman EM, Amoozegar CB, Wang T, McCaslin AF, Bouchard MB, Mansfield J, Levenson RM. 2011. In vivo optical imaging and dynamic contrast methods for biomedical research. *Phil Trans A Math Phys Eng Sci* 369: 4620–4643.
- Hogemann-Savellano D, Bos E, Blondet C, Sato F, Abe T, Josephson L, Weissleder R, Gaudet J, Sgroi D, Peters PJ, et al. 2003. The transferrin receptor: A potential molecular imaging marker for human cancer. *Neoplasia* 5: 495–506.
- Iordanova B, Ahrens ET. 2012. In vivo magnetic resonance imaging of ferritin-based reporter visualizes native neuroblast migration. *NeuroImage* 59: 1004–1012.
- Jamin Y, Gabellieri C, Smyth L, Reynolds S, Robinson SP, Springer CJ, Leach MO, Payne GS, Eykyn TR. 2009. Hyperpolarized ¹³C magnetic resonance detection of carboxypeptidase G2 activity. *Magn Reson Med* 62: 1300–1304.
- Jenkins DE, Oei Y, Hornig YS, Yu SF, Dusich J, Purchio T, Contag PR. 2003. Bioluminescent imaging (BLI) to improve and refine traditional murine models of tumor growth and metastasis. *Clin Exp Metastasis* 20: 733–744.
- Kaida A, Sawai N, Sakaguchi K, Miura M. 2011. Fluorescence kinetics in HeLa cells after treatment with cell cycle arrest inducers visualized with FUCCI (fluorescent ubiquitination-based cell cycle indicator). *Cell Biol Int* 35: 359–363.
- Kedrin D, Gligorijevic B, Wyckoff J, Verkhusha VV, Condeelis J, Segall JE, van Rhee J. 2008. Intravital imaging of metastatic behavior through a mammary imaging window. *Nat Methods* 5: 1019–1021.
- Kesarwala AH, Prior JL, Sun J, Harpstrite SE, Sharma V, Piwnica-Worms D. 2006. Second-generation triple reporter for bioluminescence, micro-positron emission tomography, and fluorescence imaging. *Mol Imaging* 5: 465–474.
- Kienast Y, von Baumgarten L, Fuhrmann M, Klinkert WE, Goldbrunner R, Herms J, Winkler F. 2010. Real-time imaging reveals the single steps of brain metastasis formation. *Nat Med* 16: 116–122.
- Kim SB, Sato M, Tao H. 2009. Split Gaussia luciferase-based bioluminescence template for tracing protein dynamics in living cells. *Anal Chem* 81: 67–74.
- Kim JB, Urban K, Cochran E, Lee S, Ang A, Rice B, Bata A, Campbell K, Coffee R, Gorodinsky A, et al. 2010. Non-invasive detection of a small number of bioluminescent cancer cells in vivo. *PLoS ONE* 5: e9364.
- Kinahan PE, Hasegawa BH, Beyer T. 2003. X-ray-based attenuation correction for positron emission tomography/computed tomography scanners. *Semin Nucl Med* 33: 166–179.
- Kirsch DG, Dinulescu DM, Miller JB, Grimm J, Santiago PM, Young NP, Nielsen GP, Quade BJ, Chaber CJ, Schultz CP, et al. 2007. A spatially and temporally restricted mouse model of soft tissue sarcoma. *Nat Med* 13: 992–997.
- Li W, Li F, Huang Q, Shen J, Wolf F, He Y, Liu X, Hu YA, Bedford JS, Li CY. 2011. Quantitative, noninvasive imaging of radiation-induced DNA double-strand breaks in vivo. *Cancer Res* 71: 4130–4137.
- Likar Y, Dobrenkov K, Olszewska M, Vider E, Shenker L, Cai S, Pillarsetty N, Hricak H, Ponomarev V. 2008. A new acycloguanosine-specific supermutant of herpes simplex virus type 1 thymidine kinase suitable for PET imaging and suicide gene therapy for potential use in patients treated with pyrimidine-based cytotoxic drugs. *J Nucl Med* 49: 713–720.
- Liu F, Lunsford EP, Tong J, Ashitate Y, Gibbs SL, Yu J, Choi HS, Henske EP, Frangioni JV. 2012. Real-time monitoring of tumorigenesis, dissemination, and drug response in a preclinical model of lymphangioleiomyomatosis/tuberosus sclerosis complex. *PLoS ONE* 7: e38589.
- Livet J, Weissman TA, Kang H, Draft RW, Tu J, Bennis RA, Sanes JR, Lichtman JW. 2007. Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature* 450: 56–62.
- Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ. 2000. In vivo visualization of gene expression using magnetic resonance imaging. *Nat Biotechnol* 18: 321–325.
- Luker GD, Sharma V, Pica CM, Dahlheimer JL, Li W, Ochesky J, Ryan CE, Piwnica-Worms H, Piwnica-Worms D. 2002. Noninvasive imaging of protein-protein interactions in living animals. *Proc Natl Acad Sci* 99: 6961–6966.

- Lyons SK, Meuwissen R, Krimpenfort P, Berns A. 2003. The generation of a conditional reporter that enables bioluminescence imaging of Cre/loxP-dependent tumorigenesis in mice. *Cancer Res* 63: 7042–7046.
- Lyons SK, Lim E, Clermont AO, Dusich J, Zhu L, Campbell KD, Coffee RJ, Grass DS, Hunter J, Purchio T, et al. 2006. Noninvasive bioluminescence imaging of normal and spontaneously transformed prostate tissue in mice. *Cancer Res* 66: 4701–4707.
- Madsen K, Marner L, Haahr M, Gillings N, Knudsen GM. 2011. Mass dose effects and in vivo affinity in brain PET receptor studies—A study of cerebral 5-HT₄ receptor binding with [¹¹C]SB207145. *Nucl Med Biol* 38: 1085–1091.
- Massoud TF, Paulmurugan R, Gambhir SS. 2010. A molecularly engineered split reporter for imaging protein–protein interactions with positron emission tomography. *Nat Med* 16: 921–926.
- Min JJ, Gambhir SS. 2008. Molecular imaging of PET reporter gene expression. *Handb Exp Pharmacol*: 277–303.
- Mizuguchi H, Xu Z, Ishii-Watabe M, Uchida E, Hayakawa T. 2000. IRES-dependent second gene expression is significantly lower than cap-dependent first gene expression in a bicistronic vector. *Mol Ther* 1: 376–382.
- Mokrejs M, Vopalensky V, Kolenaty O, Masek T, Feketova Z, Sekyrova P, Skaloudova B, Kriz V, Pospisek M. 2006. IRESite: The database of experimentally verified IRES structures (www.iresite.org). *Nucleic Acids Res* 34: D125–130.
- Muzic RF Jr, Saidel GM, Zhu N, Nelson AD, Zheng L, Berridge MS. 2000. Iterative optimal design of PET experiments for estimating β -adrenergic receptor concentration. *Med Biol Eng Comput* 38: 593–602.
- Najjar AM, Nishii R, Maxwell DS, Volgin A, Mukhopadhyay U, Bornmann WG, Tong W, Alauddin M, Gelovani JG. 2009. Molecular-genetic PET imaging using an HSV1-*tk* mutant reporter gene with enhanced specificity to acycloguanosine nucleoside analogs. *J Nucl Med* 50: 409–416.
- Nakasone ES, Askautrud HA, Kees T, Park JH, Plaks V, Ewald AJ, Fein M, Rasch MG, Tan YX, Qiu J, et al. 2012. Imaging tumor–stroma interactions during chemotherapy reveals contributions of the microenvironment to resistance. *Cancer Cell* 21: 488–503.
- Nichols J, Jones K, Phillips JM, Newland SA, Roode M, Mansfield W, Smith A, Cooke A. 2009. Validated germline-competent embryonic stem cell lines from nonobese diabetic mice. *Nat Med* 15: 814–818.
- Nicolas G, Giovacchini G, Muller-Brand J, Forrer F. 2011. Targeted radiotherapy with radiolabeled somatostatin analogs. *Endocrinol Metab Clin North Am* 40: 187–204, ix–x.
- Okazaki F, Matsunaga N, Okazaki H, Utoguchi N, Suzuki R, Maruyama K, Koyanagi S, Ohdo S. 2010. Circadian rhythm of transferrin receptor 1 gene expression controlled by c-Myc in colon cancer-bearing mice. *Cancer Res* 70: 6238–6246.
- Pardo A, Stocker M, Kampmeier F, Melmer G, Fischer R, Thepen T, Barth S. 2012. In vivo imaging of immunotoxin treatment using Katushka-transfected A-431 cells in a murine xenograft tumour model. *Cancer Immunol Immunother* 61: 1617–1626.
- Paulmurugan R, Gambhir SS. 2003. Monitoring protein–protein interactions using split synthetic renilla luciferase protein–fragment-assisted complementation. *Anal Chem* 75: 1584–1589.
- Premisrirut PK, Dow LE, Kim SY, Camiolo M, Malone CD, Miething C, Scudippo C, Zuber J, Dickins RA, Kogan SC, et al. 2011. A rapid and scalable system for studying gene function in mice using conditional RNA interference. *Cell* 145: 145–158.
- Prutik M, Poljak-Blazi M, Jakopovic M, Tomas D, Stipancic I, Zarkovic N. 2006. Altered iron metabolism, transferrin receptor 1 and ferritin in patients with colon cancer. *Cancer Lett* 238: 188–196.
- Qin JY, Zhang L, Clift KL, Huhur I, Xiang AP, Ren BZ, Lahn BT. 2010. Systematic comparison of constitutive promoters and the doxycycline-inducible promoter. *PLoS ONE* 5: e10611.
- Rabinovich BA, Ye Y, Etto T, Chen JQ, Levitsky HI, Overwijk WW, Cooper LJ, Gelovani J, Hwu P. 2008. Visualizing fewer than 10 mouse T cells with an enhanced firefly luciferase in immunocompetent mouse models of cancer. *Proc Natl Acad Sci* 105: 14342–14346.
- Rakhra K, Bachireddy P, Zabuawala T, Zeiser R, Xu L, Kopelman A, Fan AC, Yang Q, Braunstein L, Crosby E, et al. 2010. CD4⁺ T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. *Cancer Cell* 18: 485–498.
- Ray P, Tsien R, Gambhir SS. 2007. Construction and validation of improved triple fusion reporter gene vectors for molecular imaging of living subjects. *Cancer Res* 67: 3085–3093.
- Razansky D, Deliolanis NC, Vinegoni C, Ntziachristos V. 2012. Deep tissue optical and optoacoustic molecular imaging technologies for pre-clinical research and drug discovery. *Curr Pharm Biotechnol* 13: 504–522.
- Safran M, Kim WY, Kung AL, Horner JW, DePinho RA, Kaelin WG Jr. 2003. Mouse reporter strain for noninvasive bioluminescent imaging of cells that have undergone Cre-mediated recombination. *Mol Imag* 2: 297–302.
- Sakaue-Sawano A, Kurokawa H, Morimura T, Hanyu A, Hama H, Osawa H, Kashiwagi S, Fukami K, Miyata T, Miyoshi H, et al. 2008. Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* 132: 487–498.
- Schepers AG, Snippert HJ, Stange DE, van den Born M, van Es JH, van de Wetering M, Clevers H. 2012. Lineage tracing reveals Lgr5⁺ stem cell activity in mouse intestinal adenomas. *Science* 337: 730–735.
- Schipper ML, Riese CG, Seitz S, Weber A, Behe M, Schurrat T, Schramm N, Keil B, Alfke H, Behr TM. 2007. Efficacy of 99mTc pertechnetate and 131I radioisotope therapy in sodium/iodide symporter (NIS)-expressing neuroendocrine tumors in vivo. *Eur J Nucl Med Mol Imag* 34: 638–650.
- Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. 2004. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nat Biotechnol* 22: 1567–1572.
- Shu X, Royant A, Lin MZ, Aguilera TA, Lev-Ram V, Steinbach PA, Tsien RY. 2009. Mammalian expression of infrared fluorescent proteins engineered from a bacterial phytochrome. *Science* 324: 804–807.
- Singh SP, Han L, Murali R, Solis L, Roth J, Ji L, Wistuba I, Kundra V. 2011. SSTR2-based reporters for assessing gene transfer into non-small cell lung cancer: Evaluation using an intrathoracic mouse model. *Hum Gene Ther* 22: 55–64.
- Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD, et al. 2010. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* 143: 134–144.
- Szymczak AL, Workman CJ, Wang Y, Vignali KM, Dilioglou S, Vanin EF, Vignali DA. 2004. Correction of multi-gene deficiency in vivo using a single ‘self-cleaving’ 2A peptide-based retroviral vector. *Nat Biotechnol* 22: 589–594.
- Tinkum KL, Marpegan L, White LS, Sun J, Herzog ED, Piwnicka-Worms D, Piwnicka-Worms H. 2011. Bioluminescence imaging captures the expression and dynamics of endogenous p21 promoter activity in living mice and intact cells. *Mol Cell Biol* 31: 3759–3772.
- Toyokuni S. 2009. Role of iron in carcinogenesis: Cancer as a ferrotoxic disease. *Cancer Sci* 100: 9–16.
- Uhrbom L, Nerio E, Holland EC. 2004. Dissecting tumor maintenance requirements using bioluminescence imaging of cell proliferation in a mouse glioma model. *Nat Med* 10: 1257–1260.
- Vande Velde G, Raman Rangarajan J, Vreys R, Guglielmetti C, Dresselaers T, Verhoye M, Van der Linden A, Debyser Z, Baekelandt V, Maes F, et al. 2012. Quantitative evaluation of MRI-based tracking of ferritin-labeled endogenous neural stem cell progeny in rodent brain. *NeuroImage* 62: 367–380.
- Varma D, Chandrasekaran S, Sundin LJ, Reidy KT, Wan X, Chasse DA, Nevis KR, DeLuca JG, Salmon ED, Cook JG. 2012. Recruitment of the human Cdt1 replication licensing protein by the loop domain of Hec1 is required for stable kinetochore-microtubule attachment. *Nat Cell Biol* 14: 593–603.
- Vooijs M, Jonkers J, Lyons S, Berns A. 2002. Noninvasive imaging of spontaneous retinoblastoma pathway-dependent tumors in mice. *Cancer Res* 62: 1862–1867.
- Wang W, El-Deiry WS. 2003. Bioluminescent molecular imaging of endogenous and exogenous p53-mediated transcription in vitro and in vivo using an HCT116 human colon carcinoma xenograft model. *Cancer Biol Ther* 2: 196–202.
- Weber K, Thomaschewski M, Warlich M, Volz T, Cornils K, Niebuhr B, Tager M, Lutgehetmann M, Pollok JM, Stocking C, et al. 2011. RGB marking facilitates multicolor clonal cell tracking. *Nat Med* 17: 504–509.
- Weissleder R, Simonova M, Bogdanova A, Bredow S, Enochs WS, Bogdanov A Jr. 1997. MR imaging and scintigraphy of gene expression through melanin induction. *Radiology* 204: 425–429.

- Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, Basilion JP. 2000. In vivo magnetic resonance imaging of transgene expression. *Nat Med* 6: 351–355.
- Wolf F, Li W, Li F, Li CY. 2011. Novel luciferase-based reporter system to monitor activation of ErbB2/Her2/neu pathway noninvasively during radiotherapy. *Int J Radiat Oncol Biol Phys* 79: 233–238.
- Xu H, Rice BW. 2009. In-vivo fluorescence imaging with a multivariate curve resolution spectral unmixing technique. *J Biomed Opt* 14: 064011.
- Yang M, Reynoso J, Bouvet M, Hoffman RM. 2009. A transgenic red fluorescent protein-expressing nude mouse for color-coded imaging of the tumor microenvironment. *J Cell Biochem* 106: 279–284.
- Zeng Q, Yang Z, Gao YJ, Yuan H, Cui K, Shi Y, Wang H, Huang X, Wong ST, Wang Y, et al. 2010. Treating triple-negative breast cancer by a combination of rapamycin and cyclophosphamide: An in vivo bioluminescence imaging study. *Eur J Cancer* 46: 1132–1143.
- Zhang L, Lee KC, Bhojani MS, Khan AP, Shilman A, Holland EC, Ross BD, Rehemtulla A. 2007. Molecular imaging of Akt kinase activity. *Nat Med* 13: 1114–1119.
- Zhang F, Wang W, Tsuji Y, Torti SV, Torti FM. 2008. Post-transcriptional modulation of iron homeostasis during p53-dependent growth arrest. *J Biol Chem* 283: 33911–33918.
- Zhang H, Moroz MA, Serganova I, Ku T, Huang R, Vider J, Maecke HR, Larson SM, Blasberg R, Smith-Jones PM. 2011. Imaging expression of the human somatostatin receptor subtype-2 reporter gene with ⁶⁸Ga-DOTATOC. *J Nucl Med* 52: 123–131.
- Zhou Y, Rideout WM 3rd, Zi T, Bressel A, Reddypalli S, Rancourt R, Woo JK, Horner JW, Chin L, Chiu MI, et al. 2010. Chimeric mouse tumor models reveal differences in pathway activation between ERBB family- and KRAS-dependent lung adenocarcinomas. *Nat Biotechnol* 28: 71–78.
- Zuber J, McJunkin K, Fellmann C, Dow LE, Taylor MJ, Hannon GJ, Lowe SW. 2011. Toolkit for evaluating genes required for proliferation and survival using tetracycline-regulated RNAi. *Nat Biotechnol* 29: 79–83.
- Zurkiya O, Chan AW, Hu X. 2008. MagA is sufficient for producing magnetic nanoparticles in mammalian cells, making it an MRI reporter. *Magn Reson Med* 59: 1225–1231.

Cold Spring Harbor Protocols

Imaging Mouse Cancer Models In Vivo Using Reporter Transgenes

Scott K. Lyons, P. Stephen Patrick and Kevin M. Brindle

Cold Spring Harb Protoc; doi: 10.1101/pdb.top069864

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

- [Fluorescence](#) (517 articles)
- [Fluorescent Proteins](#) (259 articles)
- [In Vivo Imaging, general](#) (168 articles)
- [Labeling for Imaging](#) (339 articles)
- [Transgenic Mice](#) (139 articles)
- [Use of Reporter Genes](#) (124 articles)
- [Visualization of Gene Expression](#) (127 articles)
- [Visualization of Proteins](#) (107 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>