

Molecular Imaging Using Bioluminescence

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Abstract: Recent progress in the development of non-invasive imaging technologies continues to strengthen the role of molecular imaging in biological research. Small animal imaging with bioluminescence has been validated recently in a variety of research models and have been shown to provide continuous quantitative monitoring of the location(s), magnitude, and time-variation of gene delivery and/or expression. This article reviews the role of *in vivo* bioluminescence imaging technologies as they have been used in imaging gene delivery and gene expression for molecular imaging applications. The studies published to date demonstrate that bioluminescence imaging tools will help to understand human diseases through small animal models.

Keywords: Molecular imaging, optical imaging, bioluminescence, reporter gene, gene expression.

1. INTRODUCTION

Recent advances in molecular/cell biology, the ability to decode entire genomes, the continuous search for new targets, the unraveling of the molecular pathways of many disease and high sensitivity multimodality imaging instrumentation have allowed interactions between these fields that extends morphological observations in living subjects to a more meaningful dimension within the context of "Molecular Medicine." The term 'molecular imaging' implies the convergence of multiple imaging techniques, molecular/cell biology, chemistry, medicine, pharmacology, medical physics, biomathematics, and bioinformatics into a new imaging paradigm.

Present imaging technologies rely mostly on non-specific morphological, physiological, or metabolic changes that differentiate pathological from normal tissue rather than identifying specific molecular events (e.g. gene expression) responsible for disease. Molecular imaging usually exploits specific molecular probes as the source of image contrast. This change in emphasis from a non-specific to a specific approach represents a significant paradigm shift, the impact of which is that imaging can now provide the potential for understanding of integrative biology, earlier detection and characterization of disease, and evaluation of treatment. Massoud and Gambhir [1] suggested several important goals in molecular imaging research, namely: (1) To develop non-invasive *in vivo* imaging methods that reflect specific molecular processes such as gene expression, or more complex molecular interactions such as protein-protein interactions; (2) To monitor multiple molecular events near-simultaneously; (3) To follow trafficking and targeting of cells; (4) To optimize drug and gene therapy; (5) To image drug effects at a molecular and cellular level; (6) To assess

disease progression at a molecular pathological level; and (7) To create the possibility of achieving all of the above goals of imaging in a rapid, reproducible, and quantitative manner, so as to be able to monitor time-dependent experimental, developmental, environmental, and therapeutic influences on gene products in the same animal or patient.

Diverse optical imaging technologies have been used to investigate molecular events in biology research which is focused on characterizing molecular interaction, signal transduction, enzyme activity, receptor/transporter status and biodistribution of various optical substrates (tracers). The underlying principles of *in vitro* optical imaging can now be tailored to *in vivo* optical imaging modalities such as bioluminescence and fluorescence imaging. The development, validation, and application of bioluminescence imaging techniques in living subjects should further enhance our understanding of disease mechanisms and go hand in hand with the development of molecular imaging [1-3].

2. Bioluminescence Imaging Technologies

Visible light can be generated in living cells using two methods: fluorescence [4] and bioluminescence [5]. For fluorescence methods, cells are labeled with dyes (for trafficking) or proteins that emit light of a limited spectrum when excited by different wavelengths of light [6]. Bioluminescence is generated by the conversion of chemical energy into visible light by enzymes generally named luciferase [5]. Fig. (1) illustrates the reaction of each bioluminescence reporter gene. The most common bioluminescence reporter genes are firefly luciferase (*fluc*) and renilla luciferase (*rluc*). Note, in general a lowercase abbreviation refers to the gene while an uppercase abbreviation refers to the protein. The *fluc* (550 aa, 62 kDa) from the North American firefly, *Photinus pyralis*, is one of the best studied luciferase due to its high quantum yield (> 88%) producing light by catalyzing the oxidation of its small molecule substrate, beetle D-luciferin (benzothiazole) in an ATP-dependent process [7]. Another well-studied luciferase, *rluc* is from the sea pansy, *Renilla reniformis* (311 aa, 34

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kDa). RLuc utilizes coelenterazine as a substrate and emit light with a peak at 480 nm, which does not require ATP. RLuc has a low enzymatic turnover and quantum yield (6%) [8]. Gaussia luciferase is from the marine copepod *Gaussia princeps*. GLuc (185 aa, 19.9 kDa) is the smallest luciferase known and is naturally secreted [9]. This luciferase emits light at a peak of 480 nm with a broad emission spectrum extending to 600 nm. RLuc and GLuc induces flash kinetics within the first 10 seconds which rapidly decays with time, whereas FLuc has glow kinetics [9, 10]. Bacterial luciferase gene (*lux*) operon encodes five gene clusters, lux C, D, A, B, E (Fig. 2A). Lux A, B control luciferase enzyme expression, while lux C, D, E control fatty aldehyde enzyme complex production which synthesize substrates. Because the *lux* operon encodes all of the proteins necessary for the function of light-emitting systems in bioluminescent bacteria,

including luciferase, substrate and substrate-regenerating enzymes, bacteria that express the *lux* operon only require oxygen but not an exogenous substrate to produce bioluminescence (Fig. 2B) [11, 12]. Characteristics of different luciferases are summarized in Table 1.

Luciferase activity in lysates from cells or tissues that suffers from the same temporal constraints can be readily measured *in vitro* using relatively inexpensive detectors based on photomultiplier tubes. Dramatic advances in low light detection technologies achieved in the last two decades resulted in the opportunity to monitor luciferase activity in living cells [13] and recently also in the living body [5]. The measurement of luciferase activity *in vivo* is based on the transmission of light through mammalian tissues and the ability to collect the transmitted light, using optical imaging devices based on highly sensitive cooled charge coupled

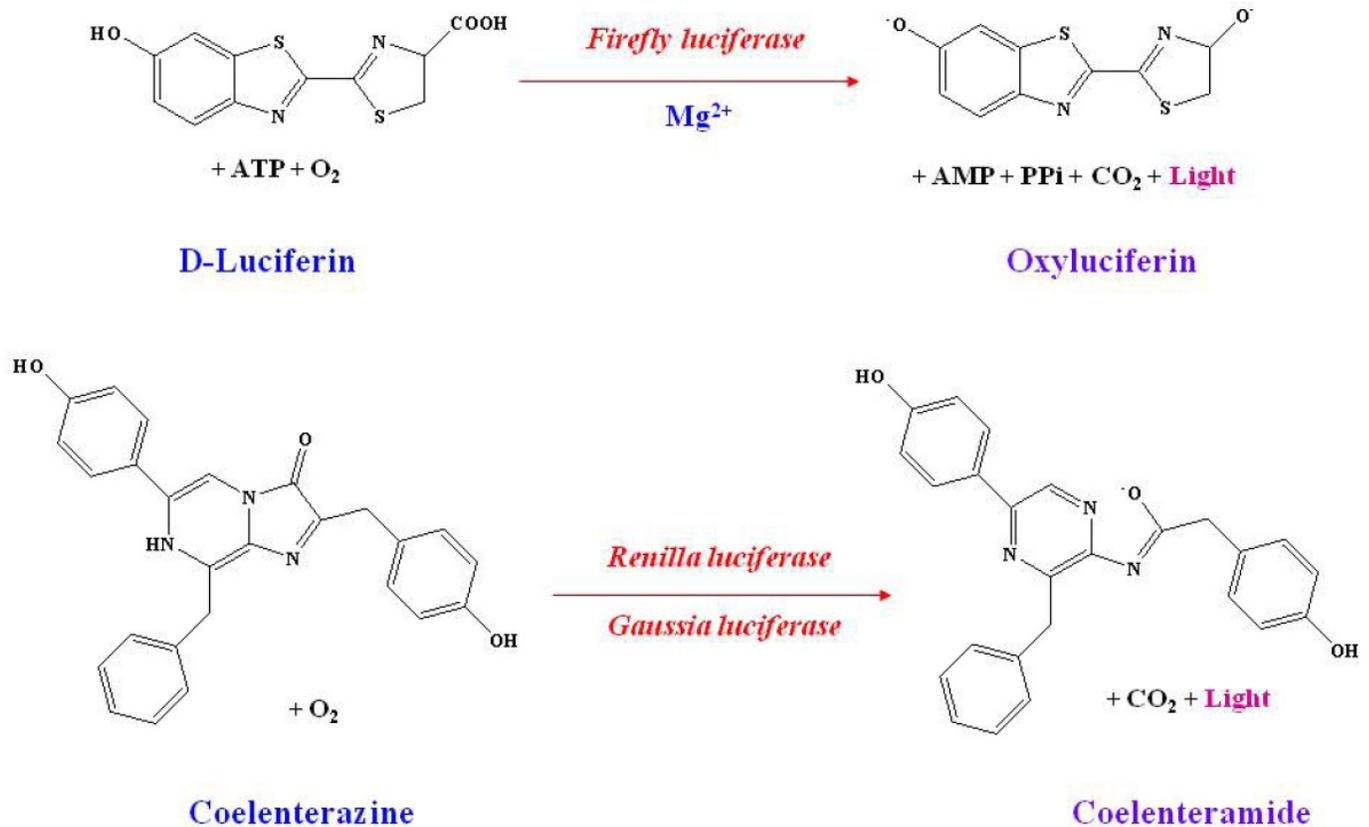


Fig. (1). Bioluminescence reaction of firefly, renilla and gaussia luciferase.

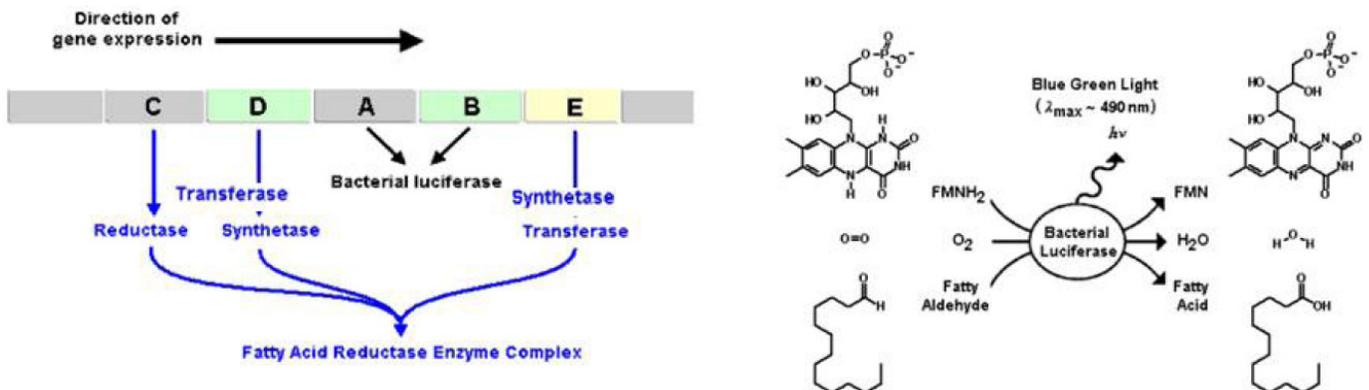


Fig. (2). Bacterial luciferase operon and bioluminescence reaction.

Table 1. Comparison of Different Luciferase

Parameter	Firefly Luciferase	Renilla Luciferase	Gaussia Luciferase	Bacterial Luciferase
Origin	Photinus pyralis	Renilla reniformis	Gaussia princeps	Photobacteria
Size	62 kDa	34 KDa	19.9 kDa	ND
Substrate	D-luciferin	Coelenterazine	Coelenterazine	Not necessary
Peak emission	562 nm	480 nm	480 ~ 600 nm	490 nm
Need cofactors (ATP, Mg ²⁺)	Yes	No	No	Yes
Eukaryotic cell expression	++	++	±	-
Bacterial expression	+	++	+	+++
Secretion from the cell	No	No	Yes	No

ND; not determined.

device (CCD) cameras [14, 15]. CCD detectors are made of silicon crystals sliced into thin sheets for fabrication into integrated circuits using similar technologies to those used in making computer silicon chips. One of the properties of silicon-based detectors is their high sensitivity to light, allowing them to detect light in the visible to near-infrared range. CCD cameras operate by converting light photons at wavelengths between 400 and 1000 nm that strike a CCD pixel with an energy of just 2-3 eV into electrons. A CCD contains semiconductors that are connected so that the output of one serves as the input of the next. Thereby, an electrical charge pattern, corresponding to the intensity of incoming photons, is read out of the CCD into an output register and amplifier at the edge of the CCD for digitization. Thermal noise is dramatically reduced if the chip is cooled; dark current falls by a factor of 10 for every 20°C decrease in temperature [15]. For bioluminescence imaging, CCD cameras are usually mounted in a light-tight specimen chamber, and are attached to a cryogenic refrigeration unit (for camera cooling to minus 90-150°C).

A key advantage of bioluminescence imaging is that no light is produced or is detectable until the substrate/enzyme interaction of a luciferase and luciferin occurs. Consequently, there is a very low background luminescence level in most animals. In contrast, the wavelengths of light used for excitation in fluorescence imaging can also excite other fluorescent molecules within tissues, and often results in high levels of background auto-fluorescence which can sometimes hinder detection of the reporter protein or dye [16]. Using either optical imaging modality, there are significant drawbacks to imaging visible light in living animals: i) there is a limited range of wavelengths (approx. 600-900nm) that can be transmitted through tissue due to light absorption by hemoglobin and water, ii) light scatter in the tissue further affects the ability to image by preventing accurate tomographic or anatomic analysis, and also requires that to image a signal from deeper within the animal, more cells must be present to produce an optical signal detectable at the surface [14]. Newer strategies are being developed in order to achieve tomography with fluorescence-based approaches [17]. There is potential for human applications with red and near-infrared light, which better penetrate tissues [18]. For instance, breast imaging, as well as

intra-operative devices, may eventually be possible with optical technologies [19]. If bioluminescence is to be utilized in humans it will require further testing of the safety of the substrates needed at high mass levels before being introduced into human subjects.

3. REPORTER GENE IMAGING

Most current application of bioluminescence reporter gene imaging in living subjects can be directed at luciferase genes externally transferred into cells of organ systems (transgenes). By adopting state-of-the-art molecular biology techniques, it is now possible to better image cellular/molecular events. In the last few years there has been a veritable explosion in the field of reporter gene imaging, with the aim of determining location, duration, and extent of gene expression within living subjects [20, 21]. Reporter genes are used to study (1) promoter/enhancer elements involved in gene expression, (2) induction of gene expression using inducible promoters, and (3) endogenous gene expression through the use of transgenes containing endogenous promoters fused to the reporter [22]. In all these cases, transcription of the reporter gene can be tracked and therefore gene expression can be studied. Unlike most conventional reporter gene methods (e.g. chloramphenicol acetyl transferase, LacZ/ β -galactosidase, alkaline phosphatase, Bla/ β -lactamase, etc. [23]) molecular imaging techniques offer the possibility of monitoring the location, magnitude, and persistence of reporter gene expression in intact living animals or humans. The reporter gene driven by a promoter of choice must first be introduced into the cells of interest. This is a common feature for all delivery vectors in a reporter gene-imaging paradigm, i.e. a complementary DNA expression cassette (an imaging cassette) containing the reporter gene of interest must be used. The promoter can be constitutive or inducible; it can also be cell-specific. If the reporter gene is transcribed, an enzyme or receptor product is made, thus trapping the imaging reporter probe, which may be a substrate for an enzyme in case of luciferase. The trapping of the probe leads to an imaging signal, be it from a photochemical reaction.

Five categories of applications for *in vivo* bioluminescence imaging will be reviewed: imaging of bacterial trafficking, gene therapies, molecular interactions, stem cell transplantation and transgenic animals.

Imaging of Bacterial Trafficking

In the 1890s, Dr. William B Coley observed that some cancer patients who developed postoperative bacterial infections experienced tumor regression and in some instances, remission from the disease. It was speculated that the stimulation of the immune system following bacterial infection would allow the immune system to tackle cancer cells more effectively [24]. However, cancer therapy employing bacteria has been only recently investigated in a systematic way. Vogelstein *et al.* [24] created a strain of *Clostridium novyi* depleted of its lethal toxin, termed *C. novyi* NT, and showed that i.v.-administered *C. novyi* NT spores germinated in the avascular regions of tumor in mice and destroyed the surrounding tumors. Attenuated *Salmonella typhimurium* strains have been shown to preferentially amplify within tumors and to express therapeutic molecules, such as prodrug-converting enzymes [25-27]. Hoffman *et al.* [28] reisolated *S. typhimurium* from the tumor after infection of human colon cancer growing in nude mice and demonstrated that the reisolated bacteria increased tumor-targeting capability *in vivo* as well as *in vitro*.

We have developed a quantitative, non-invasive imaging technique that enables monitoring of bacterial migration in living subjects (Fig. 3) [11, 12]. In our novel protocol, we used the *E. coli* K12 strain MG1655, which is regarded as the canonical *E. coli* strain. Bioluminescent bacteria were generated by transforming MG1655 with an expression plasmid (pLux) that contains the *luxCDABE* operon from *Photobacterium leognathi*. This operon encodes several proteins required for bioluminescence, including bacterial luciferase, the substrate and the substrate-regenerating enzymes. Bacteria that have been engineered to express the *Lux* operon do not require an exogenous source of substrate to produce bioluminescence. The luciferase-based approach offers several useful features: (i) it requires no excitation light, which allows the imaging the bacteria in deep tissue without the inconvenience of light scattering and attenuation through body tissues; (ii) there is no endogenous bioluminescence from mammalian cells and tissues, which results in very low background. This approach requires the long data collection times and expensive cooled CCD detector due to weak signal intensity of bioluminescence.

In the absence of selection, *E. coli* fails to maintain the pLux expression plasmid, particularly in infected animals [29]. Therefore, we employed a balanced-lethal host-vector system, which coupled the expression of the *Lux* operon to the gene for aspartate β -semialdehyde dehydrogenase (*asd*), a key enzyme in the biosynthetic pathway of diaminopimelic acid (DAP) which is an essential component of the peptidoglycan of all Gram-negative and certain Gram-positive bacteria [30]. *E. coli asd* mutants require DAP, and undergo lysis in its absence. In order to select bacteria that retained pLux *in vivo*, the chromosomal copy of *asd* was mutated in the host strain, resulting in the viability of only strains that retained the Asd^+ pLux construct. The procedure for constructing the *asd* mutant of wild-type MG1655 was based on the previously reported method of Datsenko and Wanner (2000) [31]. Using this technique we have demonstrated that this genetic engineering and imaging

protocol is a powerful approach for the quantitative visualization of the distribution of bacteria in mouse tumor models [11].

Imaging of Gene Therapies

In molecular biology, reporter genes like green fluorescent protein (*gfp*) and firefly luciferase (*fluc*) have been used to monitor the transfer and regulation of genes in cells [32]. Previously, to assay the expression of a gene, invasive techniques were used, but reporter genes have been validated that can be used in radionuclide and optical imaging modalities, to study gene expression *in vivo*. The first demonstration of cardiac reporter gene imaging in living subjects was reported with *fluc* bioluminescence imaging [33]. Recently, Gambhir group developed novel imaging approaches that allow noninvasive assessment of myocardial response to cell therapy using embryonic cardiomyoblasts expressing HSV1-sr39tk and/or FLuc [34]. The location, magnitude, and survival duration of transplanted cells were monitored noninvasively using PET and bioluminescence optical imaging.

Tumor-restricted gene expression through tissue specific transcriptional targeting is an attractive approach for gene therapy. It has been demonstrated that gene expression of highly efficient gene therapy vectors can be targeted to tumors using cell-type or tissue-type specific promoter elements. Low levels of imaging reporter gene expression owing to relatively weak tissue-specific promoters were circumvented with VP16 transactivating domains fused to yeast GAL4 DNA-binding domains. This two step transcriptional amplification (TSTA) system was valuable in demonstrating PSA (prostate-specific antigen) or CEA (carcinoembryonic antigen) driven reporter gene expression *in vivo* using *Fluc*, HSV1-sr39tk or HSV1-tk under the control of GAL4-responsive elements [35-39]. Further studies are necessary to link this system to amplify both therapeutic and reporter gene expression. These approaches hold significant promise for development of tissue specific vectors with high levels of gene expression. Further issues centered on imaging gene therapy have been reviewed by Min *et al.* [40] and Wu *et al.* [22].

Imaging of Molecular Interactions

To image protein-protein interaction in living mice, Ray *et al.* [41] have used the well studied yeast two-hybrid system adapted for mammalian cells and modified it to be inducible. They used the *NF- κ B* promoter to drive expression of two fusion proteins (VP16-MyoD and GAL4-ID), and modulated the *NF- κ B* promoter through TNF α . *Fluc* reporter gene expression was driven by the interaction of MyoD and ID through a transcriptional activation strategy. They demonstrated the ability to detect this induced protein-protein interaction in cell culture and image it in living mice by using transiently transfected cells. In a similar way, Luker *et al.* [42] engineered a fusion reporter gene comprising a mutant HSV1-tk and GFP for readout of a tetracycline-inducible, two-hybrid system *in vivo*. By using microPET, interactions between p53 tumor suppressor and the large T antigen of simian virus 40 were visualized in tumor xenografts of HeLa cells stably transfected with the imaging constructs. More recently, Paulmurugan *et al.* [43] have also

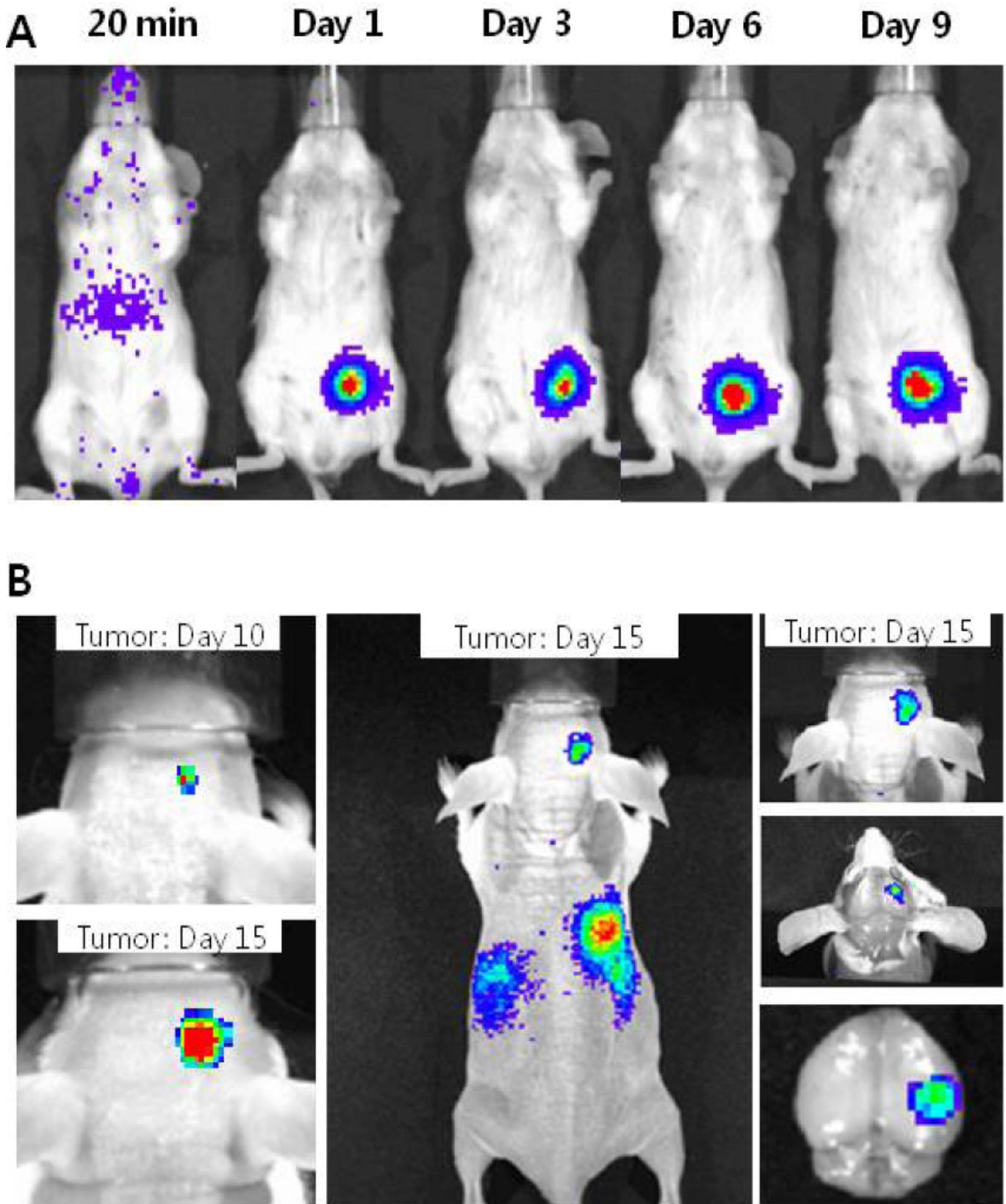


Fig. (3). Bioluminescence imaging of bacteria expressing lux operon. **(A)** *In vivo* bioluminescence imaging was performed on a BALB/c mouse with 4T1 mouse breast cancer in the left 3rd mammary gland. *E. coli* MG1655 expressing lux operon (1×10^8 CFU) were injected intravenously *via* the tail vein. **(B)** *In vivo* bioluminescence imaging was performed on a nude mouse with C6 rat glioma expressing *firefly luciferase* in the brain cortex. After 10 and 15 days of cellular inoculation, tumor growth was demonstrated by injection of D-luciferin (30 mg/kg) (left panel). Attenuated *Salmonella typhimurium* expressing lux operon (1×10^8 CFU) was injected intravenously *via* the tail vein. Bacterial luciferase signal was observed in the corresponding area of tumor growth without injection of D-luciferin (mid and right panel).

validated the use of split reporter technology to show that both complementation and intein-mediated reconstitution of FLuc can be used to also image protein-protein interactions in living mice. This approach has the advantage of potentially imaging interactions anywhere in the cell, whereas the yeast two-hybrid approaches are limited to interactions in the nucleus. Imaging interacting protein partners in living subjects could pave the way to functional proteomics in whole animals and provide a tool for evaluation of new pharmaceuticals targeted to modulate protein-protein interactions.

An important likely future possibility is the ability to image multiple molecular events in one population of cells. This may be attainable by combining two or more of the above described strategies for gene marking and imaging the trafficking of cells with those entailing linked expression of an imaging gene to an endogenous promoter, or to an exogenous therapeutic gene. As such, in these experiments it is foreseeable that one reporter may reveal the spatial distribution of cells and whether they have reached a specific target, and another reporter may indicate whether a certain gene becomes upregulated at this site or if a more complex interaction occurs. Efforts are underway to demonstrate the feasibility of this concept of simultaneous multiplexing of molecular imaging strategies, with a view to a better understanding the complexities of molecular pathways and networks.

Imaging of Stem Cell Implantation

Therapeutic effect of stem cells may be related to the secretion of multiple angiogenic cytokines by stem cells, which would contribute to the formation of a mechanical scaffold or to the recruitment of other beneficial cells to the region. However, most techniques used for the analysis of stem cell survival in animal models have relied on postmortem histology to determine the fate and migratory behavior of stem cells. This approach, however, precludes any sort of longitudinal monitoring. An approach which would allow for the monitoring of stem cell activities within the context of the intact whole-body system, rather than with histological slides, would allow us to gain further insights into the underlying biological and physiological properties of stem cells.

Several imaging strategies are currently under active investigation, including radionuclide labeling, ferromagnetic labeling, and reporter gene expression [44]. In recent years, several investigators have attempted to address this issue, using optical reporter gene expression. This approach was initially intended to allow for the serial tracking and quantification of transplanted stem cells, in a non-invasive and highly sensitive manner. During the process of reporter gene expression, the cells are transfected with reporter genes before being implanted into the myocardium [45]. In cases in which the cells remain alive, the reporter gene will be expressed. In cases in which the cells are dead, the reporter gene will not be expressed. Employing this approach, Wu *et al.* [45] used embryonic cardiomyoblasts which express HSV1-tk or FLuc reporter genes. They then noninvasively tracked them using either micro-PET or bioluminescence optical imaging. Drastic reductions were noted in signal

intensity within the first 1 to 4 days, and this was tentatively attributed to acute donor cell death as the result of inflammation, adenoviral toxicity, ischemia, or apoptosis. Kim *et al.* [46] also employed luciferase reporter gene to demonstrate migration of neural progenitor cells to the site of infarction from the contralateral parenchyma. Many of the studies would likely benefit from imaging feedback with bioluminescent imaging. Min *et al.* [47] also employed luciferase reporter gene to determine that bioluminescence imaging expedites the fast throughput screening of pharmaceutical agents, allowing for the noninvasive tracking of cord blood derived mesenchymal stem cell (CBMSC) survival within animals. In rat cardiac CBMSC transplant models, transient immunosuppressive treatment with tacrolimus or cyclosporine was shown to improve donor cell survival (Fig. 4).

Imaging of Transgenic Animals

A transgenic mouse with a specific reporter gene can be produced by injecting the gene fragment of interest into fertilized eggs and by subsequently selecting positive founders by southern blotting or genomic PCR to confirm the presence of the injected gene in the host animal genome. The merits of molecular imaging of transgenic animals can be outlined as follows: the transgenic animal in which all cells possess the reporter and specific responsive element can be used for investigating drug development [48], toxicologic analyses [49-51], cancer progression [52] and stem cell therapy [53].

The estrogen-responsive element-Luc transgenic mouse, in which all cells possess the Luc gene and estrogen receptor enhancer, is an example of reporter animal that can be used for drug development. This mouse model was specifically made to study and develop ligands that activate *via* nuclear estrogen receptor binding, can report on the generalized state of estrogen receptor activity, and be used in studies on the dynamics of estrogen receptor activity. It has been demonstrated that this model system is sensitive enough to detect responses to low, physiological concentrations of various ligands [48]. Transgenic mice have been used for to test genotoxicity [49] or to signal the presence of toxic inorganic compounds through reporter gene expression driven by hsp70 promoter [50, 51]. To evaluate prostate cancer progression in animal models, Iyer *et al.* [52] generated a transgenic mouse model of luciferase gene expression controlled by the PSA promoter in living mice using bioluminescence imaging. The bioluminescence signal in the prostate was detected as early as 3 week of age and showed prostate-specific expression. They also demonstrated that blocking androgen activity could downregulate luciferase expression in the prostate. Transgenic mice can also be used to investigate stem cell therapies. Cao *et al.* [53] produced transgenic mice expressing FLuc controlled by β -actin promoter and monitored engrafted hematopoietic stem cells in irradiated recipient mice in real time by bioluminescent imaging. After stem cells have migrated to target tissue, their differentiation to mature functional cells is critical for successful therapy. Carlsen *et al.* [54] have developed transgenic mice that expressing FLuc under the control of the nuclear factor κ B (NF- κ B) promoter, enabling real-time imaging of NF- κ B activity and its modulation in

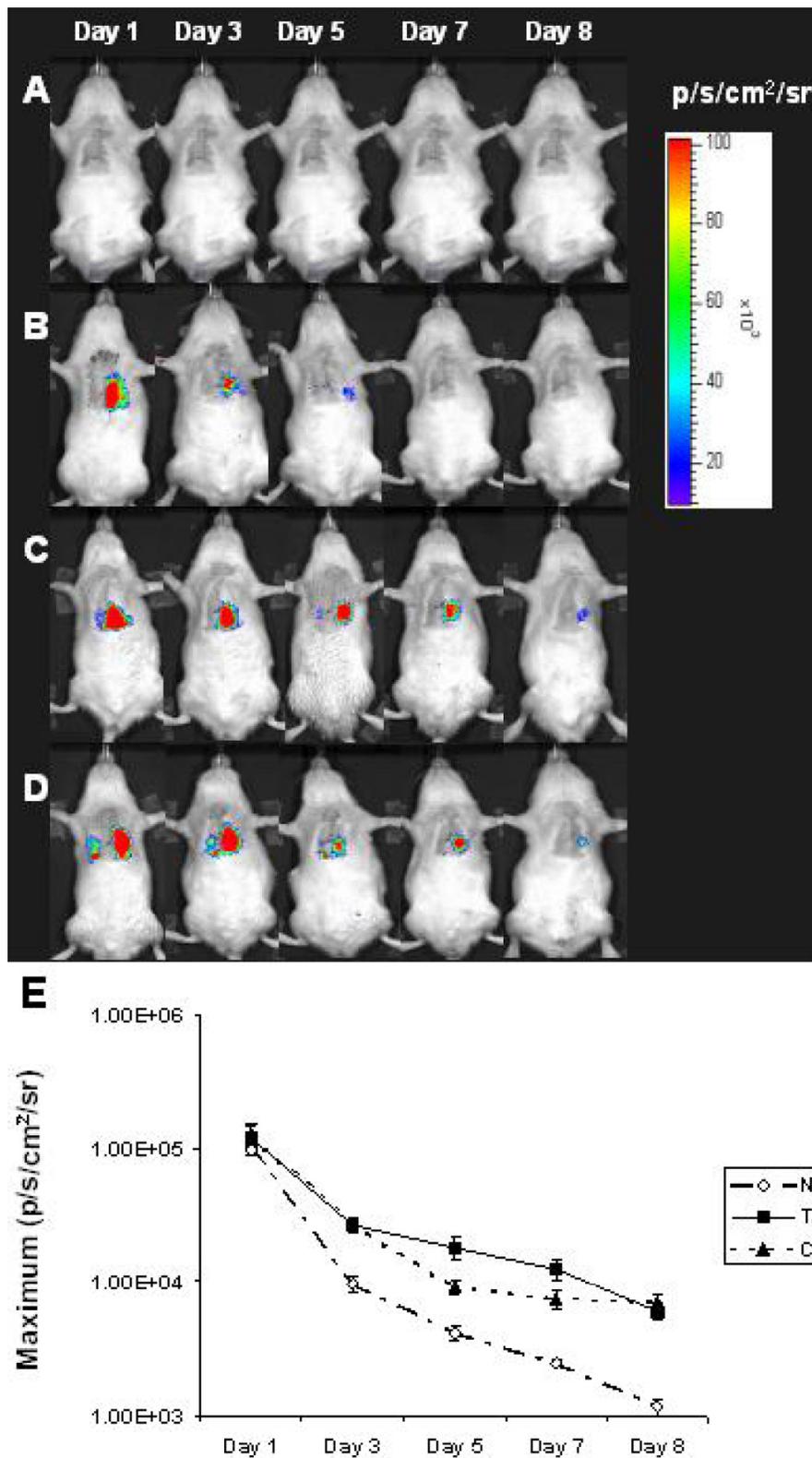


Fig. (4). Bioluminescence optical imaging of cord blood derived mesenchymal stem cell (CBMSC) transplantation in living rats. (A) Negative control rats that received saline injections into the myocardium exhibited no imaging signals coming from the anterior chest. (B) Rats were injected with CBMSCs, which expressed the firefly luciferase gene, into the anterolateral wall of the myocardium. Imaging signals were apparent from day 1. Signal intensity disappeared completely on day 7 after transplantation. (C, D) Rats were treated with tacrolimus (C, 1 mg/kg/day) or cyclosporine (D, 5 mg/kg/day) from -3 day to 8 days of transplantation. Imaging signals were apparent from day 1 to day 7. Signal intensity disappeared almost completely on the 8th day after transplantation. (E) We determined there to be a significant difference in imaging signal intensity between the non-treated group and the treated group (tacrolimus or cyclosporine) from day 3 to day 7 (reprinted with permission of [47]).

living mice. Further issues centered on imaging of transgenic animals have been reviewed by Massoud *et al.* [1] and Kang *et al.* [55].

4. CONCLUSIONS

Animal models of human diseases became a cornerstone of our knowledge of the disease mechanisms by placing target genes and processes in the appropriate physiological settings. We have obtained much of our current knowledge by monitoring obvious phenotypic changes using small animal imaging technologies. Bioluminescence imaging equipment for the analysis of a study object from multiple angles to generate three-dimensional data is now available. Moreover, the multimodal imaging instrument is a promising item to be developed since specific strengths of each modality enhance the quality of analyzing data.

Different luciferase reporters derived from *firefly*, *renilla reniformis*, *gaussia princeps* and *photobacterium* have a potential to allow multiplex multicellular imaging acquisition in a single animal using different substrate. For multicolor analysis, generation of novel luciferases with distinct wavelength of emission may be required.

The successes obtained using bioluminescence imaging strategies to study diseases will continue paving the way for basic, preclinical, and translational research. In future, we anticipate further development of these non-invasive imaging strategies for use with *in vivo* high-throughput screening, multiplexed imaging of multiple luciferases simultaneously, and combination of different imaging modalities.

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REFERENCES

- [1] Massoud TF, Gambhir SS. Molecular imaging in living subjects: Seeing fundamental biological processes in a new light. *Genes Dev* 2003; 17: 545-80.
- [2] Min JJ, Massoud TF. Recent progress in medical imaging: molecular imaging in living subjects. *J Kor Soc Med Informatics* 2004; 9: 349-73.
- [3] Min JJ. Radionuclide reporter gene imaging. *Korean J Nucl Med* 2004; 38: 143-51.
- [4] Yang M, Baranov E, Moossa AR, Penman S, Hoffman RM. Visualizing gene expression by whole-body fluorescence imaging. *Proc Natl Acad Sci USA* 2000; 97: 12278-82.
- [5] Contag PR, Olomu IN, Stevenson DK, Contag CH. Bioluminescent indicators in living mammals. *Nat Med* 1998; 4: 245-7.
- [6] Zhang J, Campbell RE, Ting AY, Tsien RY. Creating new fluorescent probes for cell biology. *Nat Rev Mol Cell Biol* 2002; 3: 906-18.
- [7] Edinger M, Hoffmann P, Contag CH, Negrin RS. Evaluation of effector cell fate and function by *in vivo* bioluminescence imaging. *Methods* 2003; 31: 172-9.
- [8] Matthews JC, Hori K, Cormier JM. Purification and properties of *Renilla reniformis* luciferase. *Biochemistry* 1977; 1: 85-91.
- [9] Tannous BA, Kim DE, Fernandez JL, Weissleder R, Breakefield XO. Condon-optimized *Gaussia* luciferase dDNA for mammalian gene expression in culture and *in vivo*. *Mol Ther* 2005; 11: 435-43.
- [10] Bhaumik S, Gambhir SS. Optical imaging of *Renilla* luciferase reporter gene expression in living mice. *Proc Natl Acad Sci USA* 2002; 99: 377-82.
- [11] Min JJ, Nguyen VH, Kim HJ, Hong Y, Choy HE. Quantitative bioluminescence imaging of tumor-targeting bacteria in living animals. *Nat Protoc* 2008; 3: 629-36.
- [12] Min JJ, Kim HJ, Park JH, *et al.* Noninvasive real-time imaging of tumors and metastases using tumor-targeting light-emitting *Escherichia coli*. *Mol Imaging Biol* 2008; 10: 54-61.
- [13] Craig FF, Simmonds AC, Watmore D, McCapra F, White MR. Membrane-permeable luciferin esters for assay of firefly luciferase in live intact cells. *Biochem J* 1991; 276: 637-41.
- [14] Rice BW, Cable MD, Nelson MB. *In vivo* imaging of light-emitting probes. *J Biomed Opt* 2001; 6: 432-40.
- [15] Spibeay CA, Jackson P, Herick K. A unique charge-coupled device/xenon arc lamp based imaging system for the accurate detection and quantitation of multicolour fluorescence. *Electrophoresis* 2001; 22: 829-36.
- [16] Billinton N, Knight AW. Seeing the wood through the trees: a review of techniques for distinguishing green fluorescent protein from endogenous autofluorescence. *Anal Biochem* 2001; 291: 175-97.
- [17] Ntziachristos V, Tung CH, Bremer C, Weissleder R. Fluorescence molecular tomography resolves protease activity *in vivo*. *Nat Med* 2002; 8: 757-60.
- [18] Sevick-Muraca EM, Houston JP, Gurfinkel M. Fluorescence-enhanced, near infrared diagnostic imaging with contrast agents. *Curr Opin Chem Biol* 2002; 6: 642-50.
- [19] Bremer C, Tung CH, Bogdanov A, Jr., Weissleder R. Imaging of differential protease expression in breast cancers for detection of aggressive tumor phenotypes. *Radiology* 2002; 222: 814-8.
- [20] Jacobs A, Heiss WD. Towards non-invasive imaging of HSV-1 vector-mediated gene expression by positron emission tomography. *Vet Microbiol* 2002; 86: 27-36.
- [21] Gambhir SS, Barrio JR, Phelps ME, *et al.* Imaging adenoviral-directed reporter gene expression in living animals with positron emission tomography. *Proc Natl Acad Sci USA* 1999; 96: 2333-8.
- [22] Wu L, Johnson M, Sato M. Transcriptionally targeted gene therapy to detect and treat cancer. *Trends Mol Med* 2003; 9: 421-9.
- [23] Spergel DJ, Kruth U, Shimshek DR, Sprengel R, Seeburg PH. Using reporter genes to label selected neuronal populations in transgenic mice for gene promoter, anatomical, and physiological studies. *Prog Neurobiol* 2001; 63: 673-86.
- [24] Agrawal N, Bettegowda C, Cheong I, *et al.* Bacteriolytic therapy can generate a potent immune response against experimental tumors. *Proc Natl Acad Sci USA* 2004; 101: 15172-7.
- [25] Zhao M, Yang M, Ma H, *et al.* Targeted therapy with a *Salmonella typhimurium* leucine-arginine auxotroph cures orthotopic human breast tumors in nude mice. *Cancer Res* 2006; 66: 7647-52.
- [26] Low KB, Itensohn M, Le T, *et al.* Lipid A mutant *Salmonella* with suppressed virulence and TNF α induction retain tumor-targeting *in vivo*. *Nat Biotechnol* 1999; 17: 37-41.
- [27] Pawelek JM, Low KB, Bermudes D. Bacteria as tumour-targeting vectors. *Lancet Oncol* 2003; 4: 548-56.
- [28] Zhao M, Geller J, Ma H, Yang M, Penman S, Hoffman RM. Monotherapy with a tumor-targeting mutant of *Salmonella typhimurium* cures orthotopic metastatic mouse models of human prostate cancer. *Proc Natl Acad Sci USA* 2007; 104: 10170-4.
- [29] Yu YA, Shabahang S, Timiryasova TM, *et al.* Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. *Nat Biotechnol* 2004; 22: 313-20.
- [30] Curtiss R, Kelly SM, Gulig PA, Nakayama K. Stable recombinant avirulent *Salmonella* vaccine strains. *Adv Exp Med Biol* 1989; 251: 33-47.
- [31] Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 2000; 97: 6640-5.
- [32] Welsh S, Kay SA. Reporter gene expression for monitoring gene transfer. *Curr Opin Biotechnol* 1997; 8: 617-22.
- [33] Wu JC, Inubushi M, Sundaresan G, Schelbert HR, Gambhir SS. Optical imaging of cardiac reporter gene expression in living rats. *Circulation* 2002; 105: 1631-4.
- [34] Wu JC, Chen IY, Sundaresan G, *et al.* Molecular imaging of cardiac cell transplantation in living animals using optical

- bioluminescence and positron emission tomography. *Circulation* 2003; 108: 1302-5.
- [35] Iyer M, Wu L, Carey M, Wang Y, Smallwood A, Gambhir SS. Two-step transcriptional amplification as a method for imaging reporter gene expression using weak promoters. *Proc Natl Acad Sci USA* 2001; 98: 14595-600.
- [36] Qiao J, Doubrovin M, Sauter BV, *et al.* Tumor-specific transcriptional targeting of suicide gene therapy. *Gene Ther* 2002; 9: 168-75.
- [37] Zhang L, Johnson M, Le KH, *et al.* Interrogating androgen receptor function in recurrent prostate cancer. *Cancer Res* 2003; 63: 4552-60.
- [38] Zhang L, Adams JY, Billick E, *et al.* Molecular engineering of a two-step transcription amplification (TSTA) system for transgene delivery in prostate cancer. *Mol Ther* 2002; 5: 223-32.
- [39] Sato M, Johnson M, Zhang L, *et al.* Optimization of adenoviral vectors to direct highly amplified prostate-specific expression for imaging and gene therapy. *Mol Ther* 2003; 8: 726-37.
- [40] Min JJ, Gambhir SS. Noninvasive imaging of gene therapy in living subjects. *Gene Ther* 2004; 11: 115-25.
- [41] Ray P, Pimenta H, Paulmurugan R, *et al.* Noninvasive quantitative imaging of protein-protein interactions in living subjects. *Proc Natl Acad Sci USA* 2002; 99: 3105-10.
- [42] Luker GD, Sharma V, Pica CM, *et al.* Noninvasive imaging of protein-protein interactions in living animals. *Proc Natl Acad Sci USA* 2002; 99: 6961-6.
- [43] Paulmurugan R, Umezawa Y, Gambhir SS. Noninvasive imaging of protein-protein interactions in living subjects by using reporter protein complementation and reconstitution strategies. *Proc Natl Acad Sci USA* 2002; 99: 15608-13.
- [44] Wu JC, Tseng JR, Gambhir SS. Molecular imaging of cardiovascular gene products. *J Nucl Cardiol* 2004; 11: 491-505.
- [45] Wu JC, Chen IY, Sundaresan G, *et al.* Molecular imaging of cardiac cell transplantation in living animals using optical bioluminescence and positron emission tomography. *Circulation* 2003; 108: 1302-5.
- [46] Kim DE, Schellingerhout D, Ishii K, Shah K, Weissleder R. Imaging of stem cell recruitment to ischemic infarcts in a murine model. *Stroke* 2004; 35: 952-7.
- [47] Min JJ, Ahn Y, Moon S, *et al.* *In vivo* bioluminescence imaging of cord blood derived mesenchymal stem cell transplantation into rat myocardium. *Ann Nucl Med* 2006; 20: 165-70.
- [48] Maggi A, Ottobrini L, Biserni A, Lucignani G, Ciana P. Techniques: reporter mice - a new way to look at drug action. *Trends Pharmacol Sci* 2004; 25: 337-42.
- [49] Gossen JA, de Leeuw WJ, Tan CH, *et al.* Efficient rescue of integrated shuttle vectors from transgenic mice: a model for studying mutations *in vivo*. *Nat Biotechnol* 1989; 8: 7971-5.
- [50] Sacco MG, Zecca L, Bagnasco L, *et al.* A transgenic mouse model for the detection of cellular stress induced by toxic inorganic compounds. *Nat Biotechnol* 1997; 15: 1392-7.
- [51] Wirth D, Christians E, Munaut C, *et al.* Differential heat shock gene hsp70-1 response to toxicants revealed by *in vivo* study of lungs in transgenic mice. *Cell Stress Chaperones* 2002; 7: 387-95.
- [52] Iyer M, Salazar FB, Lewis X, *et al.* Non-invasive imaging of a transgenic mouse model using a prostate-specific two-step transcriptional amplification strategy. *Transgenic Res* 2005; 14: 47-55.
- [53] Cao YA, Wagers AJ, Beilhack A, *et al.* Shifting foci of hematopoiesis during reconstitution from single stem cells. *Proc Natl Acad Sci USA* 2004; 101: 221-6.
- [54] Carlsen H, Moskaug JO, Fromm SH, Blomhoff R. *In vivo* imaging of NF-B activity. *J Immunol* 2002; 168: 1441-6.
- [55] Kang JH, Chung JK. Molecular-genetic imaging based on reporter gene expression. *J Nucl Med* 2008; 49: 164S-79S.

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