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Advancing animal models of neoplasia through *in vivo* bioluminescence imaging

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Abstract

Malignant disease is the final manifestation of complex molecular and cellular events leading to uncontrolled cellular proliferation and eventually tissue destruction and metastases. While the *in vitro* examination of cultured tumour cells permits the molecular dissection of early pathways in tumorigenesis on cellular and subcellular levels, only interrogation of these processes within the complexity of organ systems of the living animal can reveal the full range of pathophysiological changes that occur in neoplastic disease. Such analyses require technologies that facilitate the study of biological processes *in vivo*, and several approaches have been developed over the last few years. These strategies, in the nascent field of *in vivo* molecular and cellular imaging, combine molecular biology with imaging modalities as a means to real-time acquisition of functional information about disease processes in living systems. In this review, we will summarise recent developments in *in vivo* bioluminescence imaging (BLI) and discuss the potential of this imaging strategy for the future of cancer research.

Keywords: *In vivo*; Bioluminescence; Imaging; Tumour; Animal model

1. Introduction

The mouse's fate as the primary mammalian model in cancer research has been sealed by having a short generation time, bearing large litters, being relatively easy to breed, and more recently, by the advances in mouse genomics. The ability to manipulate the murine genome has resulted in improved models of human disease, which have led to tremendous advancements in cancer biology. Transgenic technologies, gene 'knock out' and gene 'knock in' strategies have resulted in mouse strains that have, and will, provide in-depth insight into tumorigenesis at the genetic level [1]. To gain the maximum amount of information from these sophisticated animal models, reliable and sensitive non-invasive technologies that can be applied to living mice are necessary to provide real-time read outs of cellular and molecular changes in these model systems. Significant advancements in sensitive *in vitro* analyses that utilise polymerase chain reaction (PCR)-based assays, or fluorescent cell labelling techniques have resulted in important insights into the molecular basis of malignancy. However, to accelerate the analyses of these models and to reveal changes in the context of intact organ systems further development of non-invasive *in vivo* assays that reveal cellular and molecular events with high sensitivity is essential.

Advancements in small animal imaging strategies have arisen both from modifications to structural and functional imaging modalities that are used clinically and the development of several novel modalities that are well suited for the study of animal models of human disease. The more traditional modalities that have been applied to laboratory models include ultrasound (US), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed

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tomography (SPECT), computed tomography (CT), and optical modalities that employ fluorescent indicators. *In vivo* cellular and molecular imaging strategies that are based on these modalities are relatively recent developments that have begun to reveal biological and pathophysiological changes *in vivo*; these are reviewed elsewhere [2]. One of the newer methods for *in vivo* assessment that is well suited for animal models, is accessible to investigators and offers versatility is the *in vivo* detection of bioluminescent reporter genes, that is *in vivo* bioluminescence imaging (BLI). This method allows sensitive and quantitative detection of cells non-invasively in small research animals, and is the subject of this review. This method has enabled us to non-invasively follow molecular and cellular events that lead to tumorigenesis and disease progression and rapidly reveal therapeutic efficacy in preclinical studies of novel therapeutic strategies. As such, this technological advancement can be used to greatly refine our animal models and will ultimately aid in the discovery and development of new and improved clinical cancer prevention and treatment regimens.

2. Bioluminescence imaging

Bioluminescence refers to the enzymatic generation of visible light by living organisms. Although the most commonly used bioluminescent reporter gene for research purposes has been luciferase from the North American firefly (*Photinus pyralis*; Luc), luciferase genes have been cloned from a variety of organisms, including corals (*Tenilla*), jellyfish (*Aequorea*), sea pansy (*Renilla*), several bacterial species (*Vibrio fischeri, Vibrio harveyi*), and dinoflagellates (*Gonyaulax*) [3]. Several of these genes, including that from the firefly, have been modified for optimal expression in mammalian cells and these have been used for many years in bioassays for adenosine triphosphate (ATP) quantification, and to study gene expression in transfected cells in culture [4]. This enzyme catalyses the transformation of its substrate D-luciferin (D-(−)-2-(6′-hydroxy-2′-benzothiazolyl) thiazone-4-carboxylic acid) into oxyluciferin in an ATP-dependent process, leading to the emission of photons, which can be detected using any number of low-light sensing instruments including standard luminometers. These biochemical assays are typically conducted on cell lysates; however, there are several reports of live cell assays that use luciferase [5–11].

Extending the use of luciferase as an optical reporter from biochemical and cell culture assays to living animals was dependent upon development of low light imaging systems based on charge coupled device (CCD) cameras and two other key observations. The first observation was the demonstration that the substrate for the *P. pyralis* luciferase diffuses within minutes throughout all tissues after intravenous (i.v.) or intraperitoneal (i.p.) administration and rapidly enters many cell types [12]. The second finding was that the level of photon emission and the spectrum of emitted light from Luc^+-expressing mammalian cells is adequate to penetrate tissues of small research animals, such as mice and rats, and can be detected externally with low-light imaging cameras [13]. Recent improvements in the detection technology has led to the development of *in vivo* imaging systems that employ ultra-sensitive cooled CCD cameras [14], which enabled the use of this technology in many research areas including the study of cancer [15].

Bioluminescence imaging of neoplastic tissue requires that the gene encoding the bioluminescent reporter protein be transferred to cells or tissues of interest, which can be accomplished using any number of standard gene transfer methods [15,17,18]. Cells with stable expression of luciferase can then be injected into the research animal and the light emitted from the tagged cells can be monitored externally. To generate such an image, the animals are anaesthetised and placed in a light tight chamber equipped with the CCD camera. A grayscale reference image (digital photograph) is acquired under weak illumination, then in complete darkness the photons emitted from within the body of the animal are detected externally using a range of integration times from 1 s to 5 min. The data are transferred to a computer equipped with image acquisition and analysis software for quantification [13]. To display the anatomical origin of photon emission, a pseudocolour image representing light intensity (from blue for least intense to red for most intense) is generated and superimposed over the grayscale reference image (Fig. 1). In this way cells can be localised even deep within tissues and the amount of photons detected externally can be used to estimate the number of Luc^+ cells in culture and within the animal (Fig. 1a).

3. BLI in cancer research

The first experiments using luciferase for monitoring tumour growth and response to therapy were performed by measuring luciferase activity in lysates of excised tissue [16]. However, with advancements in detectors and *in vivo* methodology, it is now possible to quantitatively examine tumour growth and regression with great sensitivity and a broad dynamic range non-invasively and *in vivo* using BLI (Fig. 1a and c; [17]). Among the earliest experiments exploring this technology were mouse xenograft models of human malignant diseases implanted in immunodeficient mice [17,18]. For this purpose, cells of the human cervical carcinoma cell line, HeLa, were transfected, selected and evaluated for stable expression of a modified luciferase gene (present in the

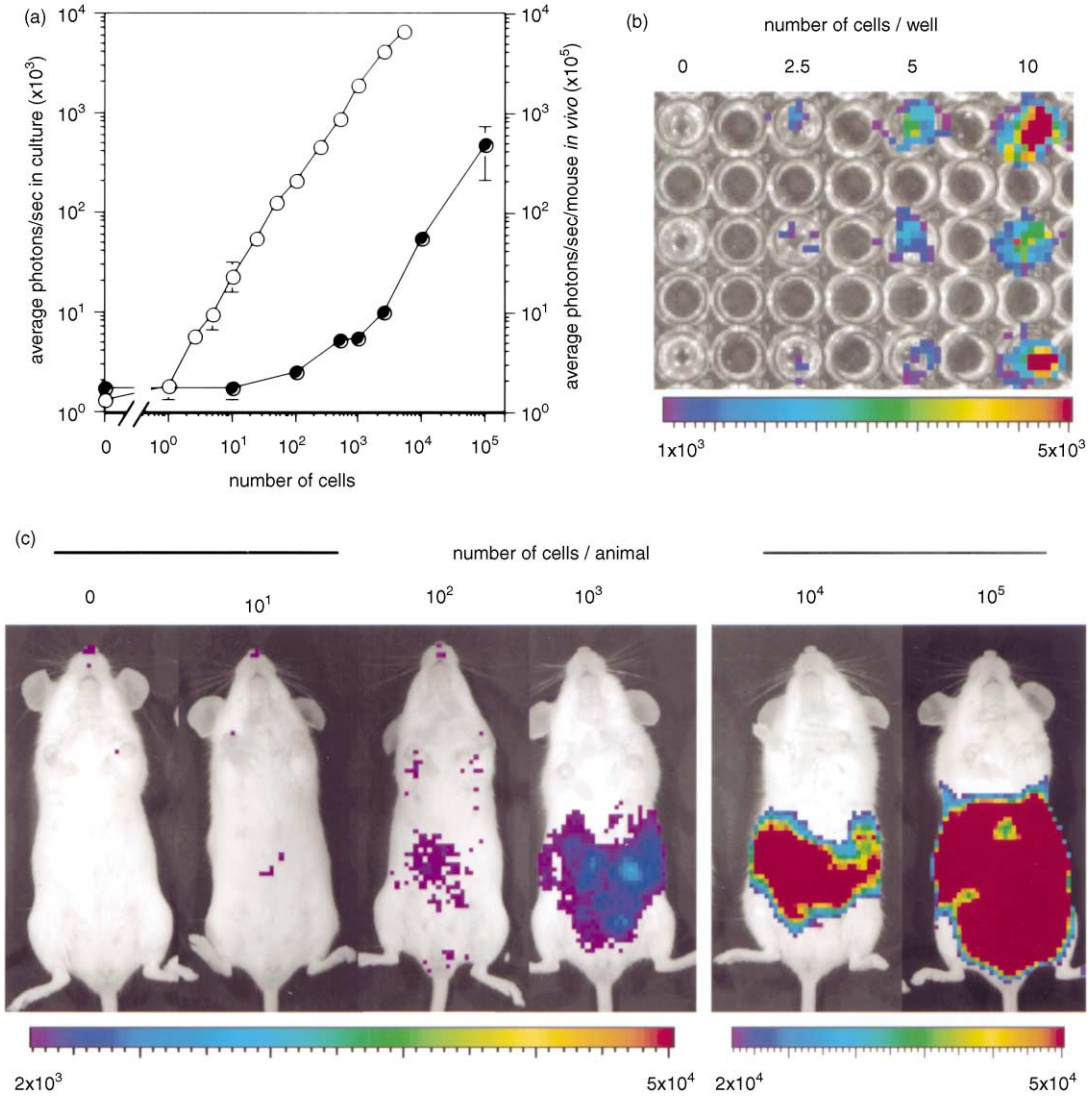

Fig. 1. Sensitivity of detecting tumour cells in culture and *in vivo*. The ability to detect small numbers of labelled cells in culture and in living animals (a) was demonstrated with a labelled human prostate cancer cell line (PC-3M-luc, clone C6). Dilutions of PC-3M-luc-C6 were plated in triplicate wells in 96-well plates (b). D-Luciferin (150 μg/ml) was added to each well and cells were imaged with a 5-min intergration time using an *In Vivo* Imaging System (IVIS, Xenogen, Alameda, CA, USA). Numbers of photons emitted per well per second were determined and plotted relative to cell numbers. As few as five cells per well produced a signal significantly above background. Similarly, dilutions of PC-3M-luc-C6 cells were injected into the peritoneal cavities of severe combined immunodeficient (SCID) mice in triplicate, D-Luciferin (150 mg/kg body weight) was administered via intraperitoneal (i.p.) injection and the animals were imaged with 5-min integration times. Images of representative animals are shown (c). The signal intensity from all three mice per group was averaged and plotted relative to cell numbers (a). As few as 100 cells produced an *in vivo* signal that was detectable above background.

pGL3 series of vectors, Promega, Madison, WI, USA). These labelled cells were injected into irradiated severe combined immunodeficient (SCID) mice via i.v. , i.p. or subcutaneous (s.c.) routes. In this model, as few as 2500 cells could be detected after i.p. injection and the intensity of the bioluminescent signal, detected externally in live animals, correlated with the number of inoculated tumour cells [17]. Since the optical reporter gene was integrated into the tumour cell genome and thereby replicated with cell division, tumour growth could be followed non-invasively by serial examination of the animals over time. These temporal measurements of tumour cell growth were quantitative and permitted spatiotemporal evaluation of tumour growth and response to both chemo- and immunotherapies [18]. Although simple in design, these initial experiments revealed the extraordinary potential of this approach and the advantages of whole body measurements of tumour growth over the more conventional vivisectionist strategies for the study of cancer.

4. Sensitivity of detection

BLI is an ideal optical approach for the study of cells *in vivo* since mammalian tissues do not emit significant levels of intrinsic bioluminescence. This inherently low background means that photon emission is restricted to cells expressing bioluminescent reporter genes and that virtually no signal is detectable from naïve cells or tissues. Moreover, luciferase and its substrate, luciferin, have not been shown to be toxic to mammalian cells, and no functional differences have been observed, thus far, between cells expressing luciferase (Luc^+) compared with parental cell lines [18]. None the less, penetration of photons through mammalian tissues is limited by both absorption and scatter [19], thus, the sensitivity of detecting internal sources of bioluminescent light has been addressed in several studies [6,17,18,20]. The sensitivity of detecting these internal sources is dependent upon many parameters including the level of Luc expression, the depth of labelled cells within the animal's body (i.e. the distance that the photons must travel through mammalian tissue) and the sensitivity of the detection system.

In its current state of development, BLI has proved to be one of the most sensitive technologies described to date for the detection of cancer cells in small research animals. In syngeneic animal models of leukaemia and lymphoma, A20 and BCL_1 tumour cells could be detected with high sensitivity in internal organs like lung, liver, spleen, lymph nodes and even within the bone marrow of BALB/c mice. As few as 1000 cells were detectable after s.c. delivery and less than 10 000 cells could be visualised in the lungs early after i.v. injection of labelled cells. Tumour infiltration of the spleen was observed earlier and with a higher sensitivity than by flow cytometry *ex vivo* after isolation of the splenocytes (M. Edinger, unpublished data). Injection of a known number of cells into the peritoneal cavity of mice has been used to determine the sensitivity and reproducibility of this approach (Fig. 1c) [17,18]. In these studies, as few as 100 cells can be detected above background (Fig. 1c).

In gene delivery experiments, Lipshutz and colleagues used a recombinant adeno-associated virus based transduction system to deliver the luciferase gene to mice *in utero*. Long-term expression of luciferase in the liver and peritoneum was observed. Limiting dilution PCR performed 8 months after the birth of the animals revealed that the signal intensity generated from 1 Luc^+ liver cell in 10^6 Luc^- liver cells was sufficient to be detected externally [6]. This demonstrated that whole body imaging is almost as sensitive as luciferase assays on tissue lysates, despite the fact that the liver absorbs light due to its high haemoglobin content [14]. In an orthotopic tumour model, Rehemtulla and coworkers used a luciferase-expressing glioblastoma cell line to show that tumour development in the rat brain could be detected by BLI and MRI with equal sensitivity [7]. Recent experiments using adenoviral-mediated gene transfer to muscle and liver revealed that, in small research animals, BLI is extremely sensitive for detecting transferred genes and may be more sensitive than PET imaging for detecting gene transfer [22].

Due to its high sensitivity, BLI will have major implications for refining animal models of human disease, for understanding the underlying biology, and accelerating the preclinical stages of drug development. Typical assays for tumour growth and response to therapeutic agents include measurement of gross tumour volume using calipers, weight loss or gain, or death of the animals. These assays have significant limitations in the study of orthotopic, metastatic and minimal disease models since they are not well suited for frequent measurements of small numbers of cells within deep tissues. Imaging, and specifically BLI, now allows tumour cell measurements early in the disease course, at stages of minimal residual disease and of metastatic lesions in living animals (Fig. 2). Use of reporter genes in transgenic strategies will facilitate the examination of animal models of spontaneous tumour development and of the mechanisms underlying tumour escape after therapy.

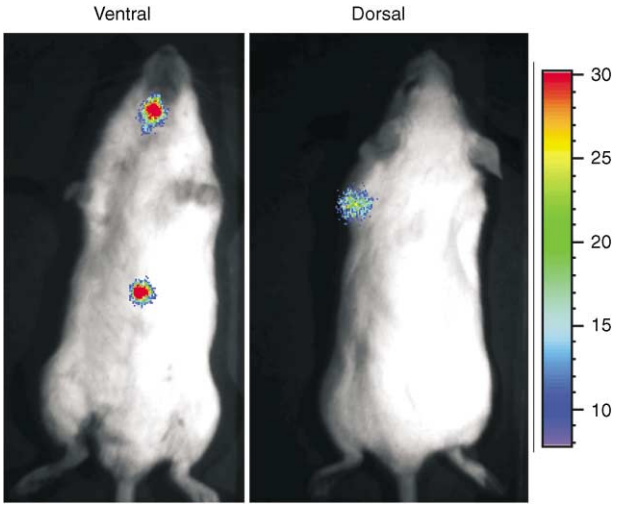

Fig. 2. Intravenous experimental metastases are detectable in xenograft models. PC-3M-luc-C6 (5×10^6 cells) were injected intravenously (i.v.) through the tail veins of male SCID-beige mice ($n=4$) and the animals were imaged weekly using an intensified charge coupled device (CCD) camera system (model C2400–32, Hamamatsu, Japan) and 5-min integration times. At day 35, post-inoculation metastases were detectable in the lungs, liver, and mandible in one of four mice (dorsal and ventral view of this mouse is shown). Lung signals without detectable metastatic lesions at other sites were apparent in 3/4 mice. The spectral sensitivity of cooled CCD cameras, as used in Figs. 1 and 3, extends past the red region of the visible spectrum and has proven to be more sensitive for detecting internal biological sources of light than the intensified CCD cameras.

5. Temporal analysis of tumour development and therapy

A significant advantage of BLI in animal models of malignant diseases is the opportunity for temporal evaluation of biological processes non-invasively (Fig. 3). Since photon emission increases in proportion to cell division, quantitative information can be generated from a single group of animals at any given time during an experiment and kinetic studies of tumour growth and regression, either spontaneously or after therapy, can be performed on groups of animals. Since the enzymatic production of light by luciferases is ATP-dependent, only metabolically active cells contribute to the signal and therefore, a decrease in signal intensity occurs as cells die. For example, growth of the luciferase expressing malignant melanoma B16F10 has been followed over time in SCID mice (Fig. 3). Light emission was detected within minutes after s.c. injection of 2×10^4 cells and the signal intensity increased as the tumour progressed and finally formed large, macroscopically visible masses. However, using the luciferase system it was possible to determine *in vivo* that central tumour necrosis occurred after 3–4 weeks, most likely due to insufficient angiogenesis. Although not detectable externally using calipers, tumour cell death was reflected by a decrease in signal intensity. The ability to detect the dynamics of tumour stasis and necrosis *in vivo* should also facilitate studies of angiogenesis inhibitors which are currently being evaluated in preclinical tumour models as well as in clinical trials [21].

Likewise, the response to chemotherapy can be monitored externally by BLI. Sweeney and colleagues [18] used chemotherapeutic agents with known differences in their efficiency against the HeLa cervical carcinoma cell line. Resistance to cyclophosphamide as well as tumour regression and eventually relapse after treatment with 5-fluorouracil and cisplatin was demonstrated *in vivo*. In this way, treatment success and cure of animals could be determined in real time, while relapses were diagnosed long before any clinical signs of disease were detectable or death of the animals occurred. Since the animals were monitored non-invasively over time, multiple data points were generated from a single experimental group, leading rapidly to statistically significant results without the necessity of serial sacrifices of groups of animals. The ability to generate more information from fewer animals, in combination with the possibility to examine many animals simultaneously with very short scanning times ranging from 1 s to 5 min, makes BLI an ideal technique for high throughput screening, for example for drug discovery and efficacy studies.

In addition to chemotherapy studies, immunotherapeutic regimens using *in vitro* stimulated and expanded human $CD8^+$ T cells which co-express the natural killer (NK) cell maker CD56 (NKT cells) were examined by BLI. NKT cells have a high cytotoxic activity against a range of tumour cell lines and primary tumour cells, both *in vitro* and *in vivo* [18,22–24]. Scheffold and colleagues demonstrated that the adoptive transfer of this cell population into SCID mice cured animals bearing a Her2/neu overexpressing human ovarian cancer (SK-OV-3), if the T cells were redirected to the tumour with a bispecific antibody binding to the CD3 T cell molecule and Her2/neu [25]. Treatment with a clinically approved anti-Her2/neu antibody (trastuzumab, Herceptin®) also cured the animals in this xenograft tumour model. Although the outcome of the two treatment modalities by traditional read-out systems was identical (long-term survival in both groups), serial imaging revealed that tumour cell clearance occurred rapidly with the T cell/antibody combination therapy (days 2–4), while trastuzumab treatment led to a gradual tumour regression over a period of weeks, suggesting that different mechanisms were responsible for the therapeutic effect [25]. These observations illustrate that BLI can be used to generate spatial information, and also reveals the kinetics of tumour growth, regression and relapse. The possibility of being able to determine the dynamics of biological processes *in vivo* is a powerful tool for many research areas. Although most malignant diseases in humans are not treated with a single chemotherapeutic agent but with combination chemotherapy, the evaluation of cytostatic drugs in preclinical trials is usually done in single agent experiments. BLI allows for the examination of combination chemotherapy regimens *in vivo*, where timing, dosage and specific combinations of drugs can be investigated to determine the ideal time intervals between treatments in animal models, which can provide insights for clinical trials.

6. Combining *in vivo* with *ex vivo* assays

Luciferase activity has been used to assess gene expression in *in vitro* assays for many years, which has resulted in an extensive body of knowledge concerning molecular and biochemical properties of the enzyme. This knowledge has facilitated the *in vivo* applications, and now links the study of biological events by *in vitro* and *in vivo* assays using a single reporter gene. Scheffold and colleagues used the light emission from the luciferase transfected human ovarian tumour cell line SK-OV-3 to establish a non-radioactive cell killing assay *in vitro* [25]. The addition of activated and expanded ($CD3^+$, $CD8^+$, $CD56^+$) NK T cells to the tumour cells in a 96-well-plate led to a dose-dependent reduction of photon emission in a 4 h cytotoxicity assay, which was further enhanced by cross-linking tumour and effector cells with a bispecific antibody against CD3 and Her2/neu [25]. Tumour cell killing as measured in this assay correlated with results generated in a standard chromium-release

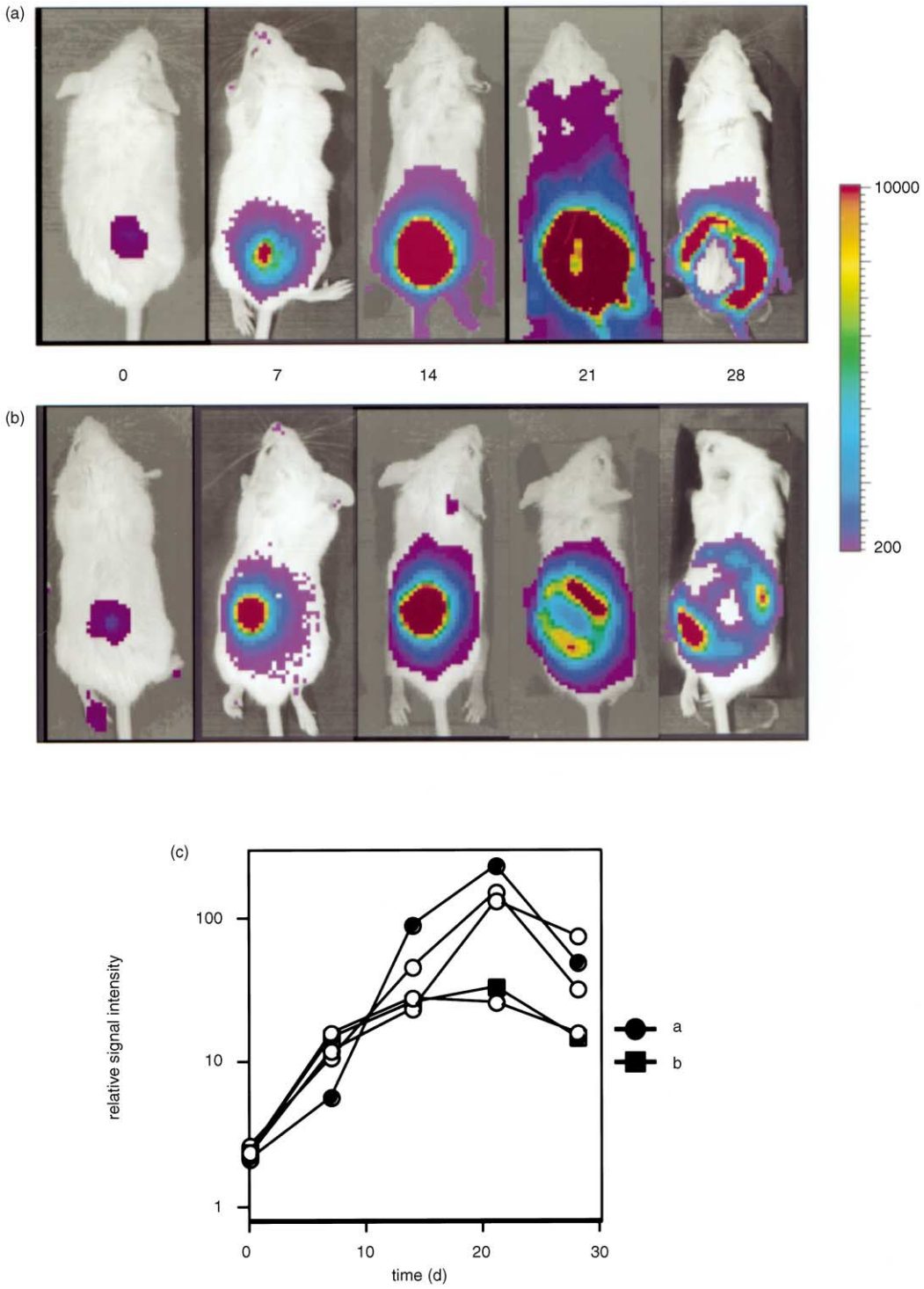

Fig. 3. Temporal analysis of tumour growth. The C57BL/6 derived B16F10 malignant melanoma cell line was transfected with a plasmid encoding firefly luciferase (pGL3 series of vectors, Promega, Madison, WI, USA) and stable lines expressing luciferase were selected. SCID mice were given 2×10^4 labelled B16F10 cells at a subcutaneous (s.c.) site, and the signals from these animals imaged at the time points indicated using a cooled CCD camera (a, b). Two representative animals are shown. The tumour signal was quantified by measuring the total counts over a 5-min integration time and plotted relative to time (c). Tumour growth as well as central tumour necrosis after 2–3 weeks could be visualised and quantified. d, days.

cytotoxicity assay and predicted the outcome of *in vivo* experiments.

BLI enables monitoring of tumour development throughout the disease course starting from minimal to late stage disease, progression including tumour cell trafficking and development of metastases can be visualised (Fig. 2 and M. Edinger, unpublished data), which is otherwise difficult to study since very few cells are present at any number of tissue sites. This allows for the localisation and measurement of tumour growth at distant sites of disease prior to sacrificing the experimental animal. Thus, the more labour-intensive histological examination, which is otherwise prone to missing sites of disease, can be directed to key target tissues. With bioluminescent reporter genes in the tumour cells, it is also possible to confirm that the lesion was recovered as the labelled cells will continue to emit light after removal from the animal. In this way, *in vivo* measurements facilitate and direct *ex vivo* assays. This will be particularly useful for tissue recovery in studies that are aimed at examining gene expression patterns in metastatic lesions using DNA microarray technologies. Such studies are further facilitated by the use of dual optical reporter genes that we [26,27] and others [28] have recently constructed by fusing the coding sequences for bioluminescent proteins with those of fluorescent proteins. The coding regions of the green and yellow fluorescent proteins (GFP, YFP) have been fused to the luciferase gene. Bioluminescence measurements can be made *in vivo*, examination of extracted tissues by fluorescence microscopy and the re-isolation of labelled cells by flow cytometry are enabled by the fluorescent protein [26,27]. Experiments examining the trafficking pattern of antigen-specific T cells in animal models of autoimmune encephalomyelitis and arthritis have already revealed the strength of this approach [26,27].

7. Limitations of BLI

The emission spectrum of the firefly luciferase is very broad with an emission peak at approximately 560 nm and a large component above 600 nm; a region of the visible spectrum where tissue penetration is high due to relatively lower absorption by haemoglobin (a primary absorber in tissues). However, the signal intensity from a depth of 1 cm is attenuated by a factor of approximately 10^{-2} for wavelengths at approximately 650 nm due to the effects of scattering [14], which may restrict the use of BLI to small research animals like mice and rats, or to superficial tissue sites in larger animals. Since light is scattered due to changes in the refractive index at cell membranes and organelles, the spatial resolution of BLI is low in comparison to imaging modalities that use more penetrating radiation such as CT, PET and SPECT. However, provided that the signal intensity from a specific anatomical location is higher than the signal from surrounding tissues, the anatomical origin can be determined, at present, down to the resolution of individual lymph nodes (1 mm), and obtaining multiviews of a single animal improves the tissue localisation. Multiview bioluminescent imaging systems currently under development will greatly improve the anatomical localisation of labelled cells.

Among the absorbing molecules in tissues is melanin in the skin, which in mammals is protective against harmful radiation, but by a similar mechanism attenuates the penetration of light from within the animal. Therefore, the sensitivity of detection in black mice is significantly reduced compared with white mice. In addition, hair and fur scatter light and thus, albino nude animals may permit the greatest resolution and sensitivity in BLI [29].

8. Future developments

BLI in living animals has already been proven to reveal exciting new insights in cancer research despite its relatively recent addition to the imaging armamentarium, and only the initial steps have been made to explore the potential of this new technology. In the very near future, it is likely that a significant broadening in the application of BLI will occur as well as further refinements in imaging technology.

Our understanding of human cancer biology has been significantly enhanced by the study of mouse models where the overexpression of oncogenes or the inactivation of tumour suppressor genes leads to tumour development [1]. Technologies allowing for the inducible expression of transgenes have further advanced our understanding in this area [30–34]. Since BLI was initially used for gene expression studies *in vitro* [35] and has already proven to be valuable for monitoring gene expression *in vivo* [12], it is anticipated that this concept will soon be applied to molecular cancer research. The integration of BLI into transgenic and knockout animals will dramatically facilitate studies of carcinogenesis, since spatial and temporal information about abnormal molecular events will be generated in real time, allowing for insights into disease development in a preclinical stage on a molecular level.

Since each of the modern molecular imaging technologies like PET, SPECT, MRI and fluorescence imaging has its specific advantages and weaknesses, the use of multifunctional reporter genes that link two or more modalities can be highly beneficial. As described above, fusion genes for bioluminescent and fluorescent imaging are already available [28]. Similarly, dual reporters for combining nuclear imaging techniques (PET) and fluorescence for gene expression studies have been developed [36]. As these technologies mature and

become more broadly accessible, many new reporter combinations and dual function instruments will be explored to optimise the read-out strategy for experimental settings.

To date, the modified luciferase gene from *P. pyralis* has been used for the establishment of bioluminescence imaging in mammals. However, luciferases comprise a whole family of photoproteins that use different substrates and emit light of varying wavelengths. This offers the possibility of developing multiple bioluminescent reporter genes to examine different cell populations or gene expression patterns at the same time within a single animal. For reporter genes using different substrates, the reporter gene expression would be examined after administration of the appropriate substrate. If reporter genes with different spectral characteristics were used, optical filters at the CCD camera may be able to discriminate them externally, provided that the light is of a wavelength that penetrates mammalian tissues; that is, both reporters emitting above 600 nm.

At the present time, BLI using CCD cameras is two-dimensional and orientation in the third dimension may be achieved, as in other modalities, by imaging animals from multiple angles leading eventually to the generation of three-dimensional or even tomographic information.

9. Summary and conclusion

In vivo imaging of bioluminescent reporter proteins is broadly applicable in cancer research and has a series of advantages compared with traditional animal models of human biology and disease. As the whole field of cellular and molecular imaging progresses, real time information about complex pathophysiological processes in living organisms will become accessible, which will further improve our understanding of the underlying biology and result in improved disease prevention and treatment regimens.

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References

1. Resor L, Bowen TJ, Wynshaw-Boris A. Unraveling human cancer in the mouse: recent refinements to modeling and analysis. *Hum Mol Genet* 2001, **10**, 669–675.
2. Weissleder R, Mahmood U. Molecular imaging. *Radiology* 2001, **219**, 316–333.
3. Hastings JW. Chemistries and colors of bioluminescent reactions: a review. *Gene* 1996, **173**, 5–11.
4. Wood KV. Marker proteins for gene expression. *Curr Opin Biotechnol* 1995, **6**, 50–58.
5. Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y. 'Green mice' as a source of ubiquitous green cells. *FEBS Lett* 1997, **407**, 313–319.
6. Lipshutz GS, Gruber CA, Cao Y, Hardy J, Contag CH, Gaensler KM. In utero delivery of adeno-associated viral vectors: intraperitoneal gene transfer produces long-term expression. *Mol Ther* 2001, **3**, 284–292.
7. Rehemtulla A, Stegman LD, Cardozo SJ, *et al*. Rapid and quantitative assessment of cancer treatment response using in vivo bioluminescence imaging. *Neoplasia* 2000, **2**, 491–495.
8. Colin M, Moritz S, Schneider H, Capeau J, Coutelle C, Brahimi-Horn MC. Haemoglobin interferes with the ex vivo luciferase luminescence assay: consequence for detection of luciferase reporter gene expression in vivo. *Gene Ther* 2000, **7**, 1333–1336.
9. Francis KP, Joh D, Bellinger-Kawahara C, Hawkinson MJ, Purchio TF, Contag PR. Monitoring bioluminescent Staphylococcus aureus infections in living mice using a novel luxABCDE construct. *Infect Immun* 2000, **68**, 3594–3600.
10. Lee SH, Camilli A. Novel approaches to monitor bacterial gene expression in infected tissue and host. *Curr Opin Microbiol* 2000, **3**, 97–101.
11. Loimaranta V, Tenovuo J, Koivisto L, Karp M. Generation of bioluminescent Streptococcus mutans and its usage in rapid analysis of the efficacy of antimicrobial compounds. *Antimicrob Agents Chemother* 1998, **42**, 1906–1910.
12. Contag CH, Spilman SD, Contag PR, *et al*. Visualizing gene expression in living mammals using a bioluminescent reporter. *Photochem Photobiol* 1997, **66**, 523–531.
13. Contag CH, Contag PR, Mullins JI, Spilman SD, Stevenson DK, Benaron DA. Photonic detection of bacterial pathogens in living hosts. *Mol Microbiol* 1995, **18**, 593–603.
14. Rice BW, Cable MD, Nelson MB. In vivo imaging of light-emitting probes. *J. Biomed. Opt*. **6**(4), 432–440.
15. Contag CH, Jenkins D, Contag PR, Negrin RS. Use of reporter genes for optical measurements of neoplastic disease in vivo. *Neoplasia* 2000, **2**, 41–52.
16. Zhang L, Hellstrom KE, Chen L. Luciferase activity as a marker of tumor burden and as an indicator of tumor response to antineoplastic therapy in vivo. *Clin Exp Metastasis* 1994, **12**, 87–92.
17. Edinger M, Sweeney TJ, Tucker AA, Olomu AB, Negrin RS, Contag CH. Noninvasive assessment of tumor cell proliferation in animal models. *Neoplasia* 1999, **1**, 303–310.
18. Sweeney TJ, Mailander V, Tucker AA, *et al*. Visualizing the kinetics of tumor-cell clearance in living animals. *Proc Natl Acad Sci USA* 1999, **96**, 12044–12049.
19. Tuchin V. Laser light scattering in biomedical diagnostics and therapy. *J Laser Appl* 1993, **5**, 43–60.
20. Wu JC, Sundaresan G, Iyer M, Gambhir SS. Noninvasive optical imaging of firefly luciferase reporter gene expression in skeletal muscles of living mice. *Mol Ther* 2001, **4**, 297–306.
21. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000, **407**, 249–257.
22. Lu PH, Negrin RS. A novel population of expanded human CD3+CD56+ cells derived from T cells with potent in vivo antitumor activity in mice with severe combined immunodeficiency. *J Immunol* 1994, **153**, 1687–1696.
23. Hoyle C, Bangs CD, Chang P, Kamel O, Mehta B, Negrin RS. Expansion of Philadelphia chromosome-negative CD3(+) CD56(+) cytotoxic cells from chronic myeloid leukemia patients:

in vitro and in vivo efficacy in severe combined immunodeficiency disease mice. *Blood* 1998, **92**, 3318–3327.
24. Baker J, Verneris MR, Ito M, Shizuru JA, Negrin RS. Expansion of cytolytic CD8(+) natural killer T cells with limited capacity for graft-versus-host disease induction due to interferon gamma production. *Blood* 2001, **97**, 2923–2931.
25. Scheffold C, Scheffold YC, Kornacker M, Contag CH, Negrin RS. Real time kinetics of HER-2/new targeted cell therapy in living animals. *Can Res*, in press.
26. Nakajima A, Seroogy CM, Sandora MR, *et al*. Antigen-specific T cell-mediated gene therapy in collagen-induced arthritis. *J Clin Invest* 2001, **107**, 1293–1301.
27. Costa GL, Sandora MR, Nakajima A, *et al*. Adoptive immunotherapy of experimental autoimmune encephalomyelitis via t cell delivery of the IL-12 p40 subunit. *J Immunol* 2001, **167**, 2379–2387.
28. Day RN, Kawecki M, Berry D. Dual-function reporter protein for analysis of gene expression in living cells. *Biotechniques* 1998, **25**, 848–850, 852–854, 856.
29. Rocchetta HL, Boylan CJ, Foley JW, *et al*. Validation of a noninvasive, real-time imaging technology using bioluminescent Escherichia coli in the neutropenic mouse thigh model of infection. *Antimicrob Agents Chemother* 2001, **45**, 129–137.
30. Sauer B. Inducible gene targeting in mice using the Cre/lox system. *Methods* 1998, **14**, 381–392.
31. Kistner A, Gossen M, Zimmermann F, *et al*. Doxycycline-mediated quantitative and tissue-specific control of gene expression in transgenic mice. *Proc Natl Acad Sci USA* 1996, **93**, 10933–10938.
32. Berns A. Turning on tumors to study cancer progression. *Nat Med* 1999, **5**, 989–990.
33. Ewald D, Li M, Efrat S, *et al*. Time-sensitive reversal of hyperplasia in transgenic mice expressing SV40 T antigen. *Science* 1996, **273**, 1384–1386.
34. Chin L, Tam A, Pomerantz J, *et al*. Essential role for oncogenic Ras in tumour maintenance. *Nature* 1999, **400**, 468–472.
35. Engebrecht J, Simon M, Silverman M. Measuring gene expression with light. *Science* 1985, **227**, 1345–1347.
36. Doubrovin M, Ponomarev V, Beresten T, *et al*. Imaging transcriptional regulation of p53-dependent genes with positron emission tomography in vivo. *Proc Natl Acad Sci USA* 2001, **98**, 9300–9305.