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Optical imaging: bacteria, viruses, and mammalian cells encoding light-emitting proteins reveal the locations of primary tumors and metastases in animals

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Abstract Early detection of tumors and their metastases is crucial for the prognosis of cancer treatment. Traditionally, tumor detection is achieved by various methods, including magnetic resonance imaging and computerized tomography. With the recent cloning, cellular expression, and real-time imaging of light-emitting proteins, such as *Renilla* luciferase (Ruc), bacterial luciferase (Lux), firefly luciferase (Luc), green fluorescent protein (GFP), or Ruc-GFP fusion protein, significant efforts have been focused on using these marker proteins for tumor detection. It has also been demonstrated that certain bacteria, viruses, and mammalian cells (BVMC), when administered systemically, are able to gain entry and replicate selectively in tumors. In addition, many tissue/tumor specific promoters have been cloned which allow transgene expression specifically in tumor tissues. Therefore, when light-emitting protein encoded BVMC are injected systemically into rodents, tumor-specific marker gene expression is achieved and is detected in real time based on light emission. Consequently, the locations of primary tumors and previously unknown metastases in animals are revealed *in vivo*. In the future it will likely be feasible to use engineered light-emitting BVMC as probes for tumor detection and as gene-delivery vehicles *in vivo* for cancer therapy.

Keywords Optical imaging · Tumors · Metastases · Luciferase · Green fluorescent protein · Bacteria · Vaccinia virus · Mammalian cells

Introduction

Successful and early tumor detection is the first important step toward cancer diagnosis and treatment. The most widely used tumor detection methods in patients are magnetic resonance imaging (MRI), computerized tomography (CT), and positron-emission tomography (PET). More recently, with the advances in cell and molecular biology, novel tumor detection methods are being proposed. Examples of such methods are the use of radiolabeled antibodies [1, 2], protease-activated near-infrared fluorescent probes [3], and receptor-targeted fluorescent ligands [4]. In addition to the attractive features of these novel tumor imaging methods, there are some characteristics of them which need improvement. Difficult to improve features include limited tumor specificity of the imaging agents and the requirement of repeated administration of the imaging agents to overcome turnover and dilution effects after systemic delivery. In search of alternative and more efficient ways to image and detect tumors *in vivo*, we have noted that microorganisms, such as bacteria, viruses, and fungi, are present in tumor tissues excised from human patients [5, 6, 7, 8, 9, 10, 11]. Notably, the microorganisms present in tumors are absent in the rest of the body of the tumor-bearing patient. It is generally believed that the microorganisms, viruses in particular, may trigger tumor induction [12, 13, 14, 15, 16]. However, the selective presence of microorganisms in solid tumors may also be explained by unique tumor properties that support microorganism entry and growth within the tumor. To take advantage of the entry and preferential replication of microorganisms in tumors, several laboratories have generated genetically modified microorganisms for tumor imaging and for the purpose of controlling tumor growth. In our laboratory, we took the approach of using genetically modified light-emitting microorganisms to visualize their movement from the blood stream into the tumor region and their replication in solid tumors, thereby revealing the location of tumors based on light emission. Beyond this, recent findings indicate that systemically injected mammalian cells gain entry into solid tumors in tumor-bearing

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organisms [17, 18]. Therefore, genetically-labeled mammalian cells may also be used for tumor detection and therapy. In addition to gene expression in tumors through BVMC targeting, tumor-specific gene expression can be achieved by linking the transgenes to tissue/tumor-specific promoters. The discovery of increasing numbers of tissue/tumor promoters will greatly facilitate the detection and ultimately the therapy of tumors in the future.

In this paper, we review the use of marker genes encoding light-emitting proteins, such as bacterial luciferase, firefly luciferase, *Renilla* luciferase, GFP, and Ruc-GFP fusion protein, to label BVMC individually. Such labeled organisms, when injected systemically into tumor-bearing animals, are monitored by amplified marker gene expression resulting from replication or from tissue/tumor-specific promoter activation in tumors. The locations of primary tumors and their metastases are thereby revealed in vivo.

Localization of light emission in animals injected with labeled BVMC

Fluorescent protein-mediated monitoring of gene expression in vivo has become a dominant method in real-time imaging. *Aequorea* GFP [19] is one of the most widely used fluorescent proteins and it has many useful characteristics. After optimized codon usage, GFP has been expressed in numerous prokaryotic and eukaryotic cells. It is heat stable and active as a fusion protein partner. It does not require exogenous substrate for fluorescence. In addition, different GFP variants have been developed over the years with altered fluorescence characteristics and protein stabilities [20, 21, 22]. These versatilities greatly facilitate the application of GFP in biomedical imaging.

The uses of GFP in cell biology [23], developmental biology [24], microbiology [25, 26], and protein chemistry [27] are the subject of several recent reviews. In particular, individual green fluorescent bacterial cells and mammalian cells are shown to be easily distinguishable from nonfluorescent tissues, which allows the tracing of GFP-labeled bacterial and mammalian cell movement in injected animals. It is shown that enhanced expression of GFP does not affect bacterial virulence [28, 29, 30]. The location of the labeled bacteria during the infection process can be monitored with great spatial resolution and temporal details [31, 32, 33]. Therefore, results obtained from imaging studies of bacterial infections in vivo may aid in elucidating the key molecular mechanisms during bacterium-host cell interaction [34, 35]. Besides bacteria, the *gfp* expression cassette has also been inserted into different viral genomes. Similarly to bacteria, the green fluorescence in cells as the result of GFP expression allows the tracing of viral infection and movement in animals [36]. Tissue-specific distribution patterns of different viruses delivered through various routes can be analyzed in great detail based on imaging of light emission [37, 38, 39, 40]. Furthermore, labeled mammalian cells expressing GFP can also be followed and their localization deter-

mined in live animals upon systemic delivery. A notable example is that one is able to monitor the metastasizing of cells, which are permanently transformed with *gfp* expression cassettes, into distant organs in live animals [41, 42]. With the aid of high resolution intravital videomicroscopy, Naumov et al. [43] are able to visualize such metastatic events as well as to quantify the number of metastases in real time. In addition to monitoring tumor metastases, GFP labeling has also been used to examine the tissue distribution pattern of engineered mammalian cells as delivery vehicles for gene therapy. Pastorino et al. [44] have analyzed the distribution pattern of GFP-labeled macrophages that are used to carry therapeutic genes after i.v. injection. In conclusion, due to high spatial resolution at the cellular level and because of the lack of substrate requirement, GFP fluorescence imaging becomes a powerful tool for tracing of BVMC in animals.

The cloning of structural genes encoding bacterial luciferase from *Vibrio harveyi* [45] and from *Vibrio fischerii* [46], firefly luciferase [47], and *Renilla* luciferase from *Renilla reniformis* [48] allows luciferase-mediated luminescence imaging [49, 50] to be widely used in biomedical research. All luciferases have a substrate requirement, e.g. the addition of coelenterazine for Ruc activity and D-luciferin for Luc activity. The presence of oxygen is also needed for luciferase-catalyzed oxidative reactions. Therefore, light emission can be readily detected only in live animals or in tissues exposed to the air. The advantage of luminescence over GFP fluorescence is that, with available substrate, the luminescence light (480 nm) generated does penetrate tissues of live animals. In contrast, GFP needs to be excited by exogenous blue light, which has a limited tissue penetration of approximately 2–3 mm, and therefore no fluorescence can be detected in deep tissues in intact animals.

The advantages associated with luciferase technology have been exploited by numerous laboratories during the past fifteen years. Bacteria containing luciferases have been used to monitor bacterial survival in live organisms. For example, gram-positive bacteria *Bacillus thuringiensis* carrying *xylA* promoter-*luxAB* fusion construct have been injected into the hemolymph or fed into tobacco hornworm (*Manduca sexta*) and the progression of bacterial replication in different tissues visualized in real time in live worms in the presence of decanal under a low light imager [51]. Similarly, the intramuscularly injected light-emitting *Staphylococcus aureus* carrying *luxABCDE* operon (no decanal addition required) is successfully monitored in live mice [52]. Interestingly, using luciferase-labeled *Streptococcus pneumoniae*, Echchannaoui et al. [53] are able to clearly demonstrate that mice carrying Toll-like receptor 2 mutations are more prone to develop bacterial meningitis in response to infection. Luciferase has also been used to trace viral infection in live animals. Honigman et al. [54] have linked *luc* cDNA to the minimal SV40 promoter and inserted the expression cassette into adenoviral genome. Using a sensitive cooled charged-coupled device (CCCD) camera, the temporal and spatial distribution pattern of the virus is visualized based on lu-

luciferase-mediated luminescence in mice after intravenous (i.v.) or intraperitoneal (i.p.) injection. Data from this study indicate that the liver is the primary site of viral infection, and the virus-mediated luciferase gene expression is present in the liver for at least three weeks after viral injection. The authors also noted that the substrate of firefly luciferase, D-luciferin, is found to easily penetrate various organs and cell types upon systemic injection to catalyze reactions in live animals. Even though a large amount of the substrate is required, the substrate is shown to be non-toxic to the animals. Luciferase-based light emission is also used to trace cultured mammalian cells in live animals. Cells transformed with luciferase expression constructs are implanted in animals, and the light emission is monitored noninvasively after systemic delivery of luciferase substrate [54, 55, 56, 57]. These findings demonstrate that the localization and tracing of the growth and migration of implanted cells are feasible based on light emission in living organisms [58, 59, 60]. They also open the way to monitor localization, differentiation, and survival of stem cells with medical importance.

Light-emitting bacteria signal the locations of tumors and metastases in animals

The presence of bacteria in excised tumors was first reported in the 1950s [5]. Recent studies suggest that bacteria may take advantage of nutrient-rich growth conditions and the anaerobic microenvironment found in the necrotic central portion of tumors for their replication. Pawelek and associates [61] have reported the preferential accumulation of an auxotrophic mutant strain of *Salmonella typhimurium* in tumors after i.p. injection of these bacteria into mice bearing melanoma tumors. Lemmon et al. [62] and Dang et al. [63] have attributed the preferential replication of *Clostridium spp.* in the tumor to the anaerobic environment of the tumor center. Based on their findings, all three laboratories have recognized the potential of bacteria-mediated gene product delivery systems for therapy of solid tumors. In addition to growth condition requirements for nutrient and anaerobicity, alternative explanations for selective accumulation of bacteria in tumors have been proposed. For example, it was shown that some bacteria tend to adhere to tumor cells but not to benign cells [64]. Based on data from our laboratory, we propose that the impaired lymphatic system in tumors [65] may be responsible for the lack of clearance of bacteria from tumors by the host immunosurveillance after escaping the vascular system.

With the use of light-emitting bacteria, the movement and accumulation of input bacterial cells in animals can be monitored reliably. Several laboratories have attempted to follow the fate of light-emitting bacteria in tumors. Zheng et al. [66] have i.v. injected GFP-labeled *Salmonella* into tumor-bearing mice. After analyzing the homogenates of tumor tissues and tissues of different organs, the authors note that bacterium-based GFP fluorescence is prevalent in tumors but much reduced in livers.

However, when administered orally, GFP-labeled attenuated *Salmonella* are found in homogenates of liver, spleen, intestine, kidney, and tumor [67]. We labeled bacteria (*Vibrio cholerae*, *Salmonella typhimurium*, *Escherichia coli*) with *luxCDABE* bacterial luciferase expression cassette [68], which results in continuous light emission in transformants. With the use of a low light video camera capable of detecting single photons, emission images were generated from individual tumorous animals (Fig. 1). Two days after a single i.v. administration of 1×10^7 bacteria into nu⁻/nu⁻ mice bearing subcutaneous (s.c.) C6 rat glioma tumors, light emission was detected in the tumor region only, which lasted up to several weeks until the tumorous animal had to be sacrificed. We found that eight-day (approximately 200 mm³ in size) or older glioma tumors were colonized by bacteria. Examination of fluid samples drawn directly from tumors of live animals one week after injection of bacteria confirmed the presence of large numbers of light-emitting bacteria. On the contrary, tail vein blood samples taken from the same animals showed no sign of bacteria immediately after sampling or after overnight culturing in liquid medium. None of the internal organs, including the liver and the spleen, showed the presence of bacteria based on light emission. Consistent with this observation, the light-emitting bacteria, once in the tumor, were not released at all or not released in sufficient numbers back into the circulation to be able to colonize newly implanted tumors. Interestingly, inflamed cutaneous wounds, such as the incision site and the ear tag region, initially also showed light emission. However, consistently, such light emission was proven to be short-term and lasted only for less than a week. In addition to the glioma model, similar results were obtained using nu⁻/nu⁻ mice bearing implanted MCF-7 or GI-101A [69] human breast tumors and HT1080 human

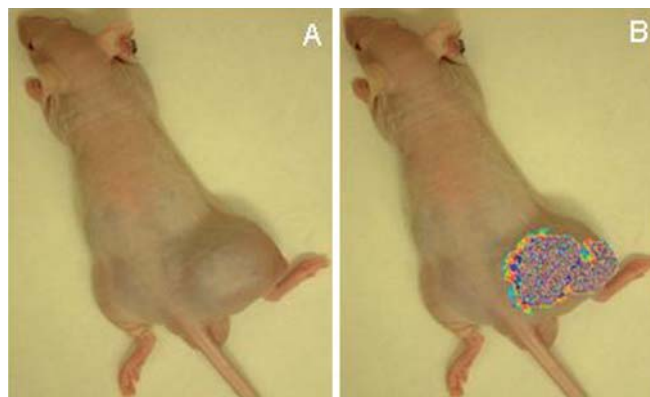


Fig. 1 Visualization of s.c. C6 glioma tumor with light-emitting *Vibrio cholerae*. Nu⁻/nu⁻ mouse bearing an 11-day old s.c. implanted C6 glioma tumor on the right lateral thigh was injected i.v. with 1×10^7 cells of *Vibrio cholerae* carrying *luxCDABE* operon sequence. Three days after injection of bacteria, the mouse was anesthetized and a photon emission image taken under a Hamamatsu ARGUS100 low light imager. The resulting photon acquisition image was superimposed onto a photographic image (panel A) of the mouse to show the location of luminescence emission specifically localized to the tumor region (panel B)

fibrosarcoma tumors. In the case of nu^{-}/nu^{-} mice bearing MCF-7 breast tumors, both the primary and the metastatic tumors were targeted and labeled by light-emitting bacteria. Similarly to nu^{-}/nu^{-} mice, C57 mice with orthotopically implanted MB-49 murine bladder tumors and Wistar rats with implanted intracranial C6 glioma tumors also showed tumor-specific bacterial replication based on imaging data. Taken together, the results showed that the ability of bacteria to find tumors in live animal models was:

1. not tumor type dependent, with no preference for fast or slow growing tumors (e.g. C6 vs. MCF-7 tumors, respectively),
2. not dependent on tumor size, since bladder tumors approximately 10 mm^3 in size were colonized just as well as glioma or breast tumors ranging from 100 to 4000 mm^3 in size, and
3. not limited to pathogenic bacteria, since *E. coli* DH5 α was also capable of finding tumors.

Bacterial imaging of tumors was successful in immunologically fully competent C57 mice and Wistar rats, as well as in immunocompromised nu^{-}/nu^{-} mice. It had been suggested earlier that anaerobic bacteria preferentially accumulate in the hypoxic necrotic center of tumors following i.v. injections [62, 63]. However, we found that *V. cholerae* and *E. coli* accumulated and replicated similarly to *Clostridium* in all tumor types tested, suggesting that the hypoxic environment is not required for bacterial survival in tumor tissues. In summary, the tumor-finding ability of bacteria combined with the luciferase imaging technology provides to the investigator a tumor detection probe with highly specific self-renewing signals able to reveal tumors and previously unknown metastases in live animals.

Viruses encoding light-emitting proteins signal the locations of tumors and metastases in animals

To obtain tumor specific gene expression, several systemic targeting strategies have been explored. These strategies include the use of tissue/tumor-specific promoters that allow the activation of gene expression only in specific organs [70, 71], the use of extracellular matrix (i.e. collagen)-targeted viral vectors [72], and the use of antibody-directed viral vectors [73, 74]. One excellent example of tumor-specific transgene overexpression through a tissue-specific promoter is demonstrated in the use of prostate-specific promoter-directed viral gene expression [75, 76]. Conditionally-replicating viruses have also been explored as tumor-specific delivery vehicles for marker genes or therapeutic genes [77, 78, 79, 80]. The best known conditionally-replicating virus is the ONYX 015 adenovirus [81], which is currently in phase III clinical trials. ONYX 015 has deletion of the E1B-55 kDa DNA fragment, which allows the replication of the virus only in cells with abnormal p53 tumor suppressor functions, e.g., in tumor cells with p53 mutations. Other examples of replication-

selective viruses include herpes simplex virus [82] and vaccinia virus (VV; [80]). It has been previously suggested that the rich availability of metabolites supports the extremely high viral titer found selectively in tumors after i.v. injection of VV with thymidine kinase-deficiency (*tk*⁻) [83]. The exact reasons for tumor-specific viral replication are not yet known. However, it is noteworthy that in our laboratory, we found that the LIVP strain of VV, which has an active *tk* gene, exhibited very limited replication in normal organs of tumor-bearing mice, but robust replication in the tumors, indicating that *tk* mutations are not required for tumor-specific entry and replication of VV.

Imaging of tumors in live animals using virus carried marker genes linked to tissue/tumor-specific promoters has been carried out in several laboratories. Adams et al. [84] have described the noninvasive detection of xenograft tumors in live mice using adenoviral vector carrying the *luc* gene fused to an enhanced prostate-specific promoter. Systemic delivery of the recombinant virus results in tumor-specific luciferase expression and light emission, which is detected by low light imaging in anesthetized animals. Interestingly, the authors claim that metastases of xenograft tumors are also detected in the lung region by the same viral vector in live animals. Lee et al. [85] link *luc* to a chimeric promoter containing prostate-specific enhancer element and then place the expression cassette into the adenovirus genome. After i.v. injection of the virus, luciferase activity is shown to be highly specific to the tumor tissue when analyzed using different tissue homogenates. In other studies, different versions of the prostate-specific promoters, such as tetracycline-regulated prostate-specific promoter [86] or androgen-responsive GAL-4-VP16-based synthetic promoter [87], have also been examined for their ability to exhibit prostate specificity and reduced activation of gene expression elsewhere in the body. Furthermore, Varda-bloom et al. [88] have constructed adenoviral vectors containing the *luc* or the *gfp* sequences linked to murine preproendothelin (PPE-1) promoter. They have successfully demonstrated the visualization of neovasculature in primary and metastatic tumors based on light emission due to endothelial cell-specific activation of the PPE-1 promoter.

Imaging of tumors is also achieved in animals by using the tumor-targeted conditionally-replicating viruses, such as VV described above. VV carrying *luc* [83] or *gfp* [80] expression cassettes constructed by Bartlett and colleagues have been injected i.v. or i.p. into tumor-bearing animals [83, 89, 90, 91]. Luciferase activity and GFP fluorescence are noted specifically in the tumors. However, in these studies, the luciferase assay and GFP fluorescence analyses are performed in vitro using homogenates of excised tumors and other organs. No attempt was made to detect luciferase-mediated luminescence activity or GFP fluorescence directly in live animals after viral injection. We have investigated the detection of tumors in live animals by genetically labeled VV using low light imaging and fluorescence microscopy. A dual reporter fusion construct encoding the Ruc-GFP fusion protein [92, 93] has been

inserted into the nonessential region of L1VP strain of VV genome by homologous recombination [94]. We first examined the VV distribution in nude mice bearing s.c. C6 glioma tumors under a fluorescence stereomicroscope. Three days after viral injection (1×10^8 pfu), tumor-specific GFP light emission was detected in tumors ranging from 25 to 4000 mm³ in size. The GFP signal lasted for up to 45 days, indicating continued viral replication and gene expression. Systemic delivery of Ruc substrate coelenterazine to live animals also confirmed the presence of strong luminescence originating only from the tumor region. Close examination of live animals revealed clusters of green fluorescent patches over the tumor surface. Overtime, the fluorescent patches turned into expanding fluorescent rings, indicating the spread of infection to neighboring tumor cells. Cross sections of the tumors indicated that the green patches were at the periphery of the tumors where the fast-dividing cells reside. This finding was in clear contrast to tumors labeled with bacteria, where the emitted light was mostly concentrated in the necrotic center of the tumor. We also found that the earliest time point when VV-derived GFP fluorescence was noted was in 5-day-old glioma tumors after s.c. implantation of 5×10^5 cells with a volume of approximately 25 mm³. This detection ability was significantly sooner and the tumor size was significantly smaller than those required for bacteria-mediated

detection of glioma tumors. These data imply that VV may utilize a somewhat different entry and survival mechanism in tumors than bacteria do. The tumor-specific infection and labeling by VV based on Ruc-GFP fusion protein fluorescence was also observed in nude mice carrying implanted PC-3 human prostate tumors, GI-101A (Fig. 2) and MCF-7 human breast tumors, and in C57 mice with orthotopically implanted MB-49 murine bladder tumors. In the case of MCF-7 breast tumor models, both the primary tumors implanted in the mammary fat pad and the metastatic tumors on the surface of the chest and on the surface of the lung were detected by GFP imaging. Amazingly, metastatic tumors as small as 0.5 mm in diameter on the surface of the lung were found using the light-emitting VV. In conclusion, these data showed that genetically labeled VV can be used to detect primary and metastatic tumors in live animals by real-time optical imaging. Furthermore, since the VV-mediated GFP expression is observed only in tumor cells, GFP fluorescence may also be useful as a potential marker for identifying tumor margins to facilitate precise tumor removal during surgery in the future.

Can light-emitting mammalian cells also reveal the location of tumors and metastases in a living organism?

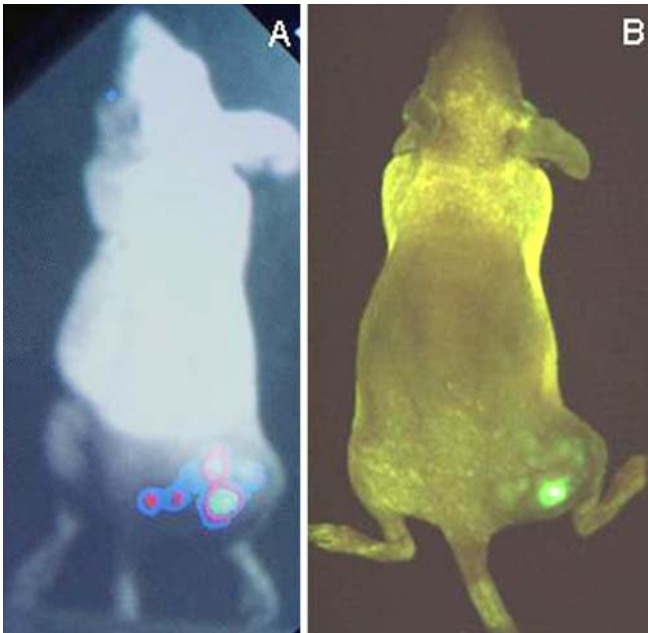
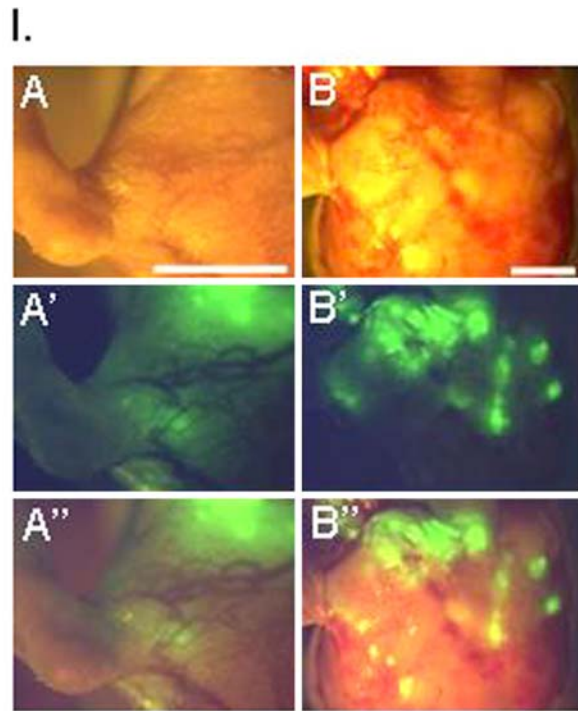


Fig. 2 Visualization of s.c. GI-101A breast tumor with labeled VV. A female nu/nu- mouse bearing a 30-day old s.c. implanted GI-101A human breast tumor was i.v. injected with 1×10^7 pfu of VV carrying *ruc-gfp* fusion expression cassette. Nine days after viral delivery, 10 µg of coelenterazine in 200 µL of phosphate-buffered saline was i.v. injected to the animal. Under anesthesia, whole-body optical imaging of the animal was performed using the ARGUS100 low light imager and Lighttools fluorescence macro-imaging system. Tumor-specific luminescence light emission (panel A) and GFP fluorescence (panel B) were noted in the xenograft tumor

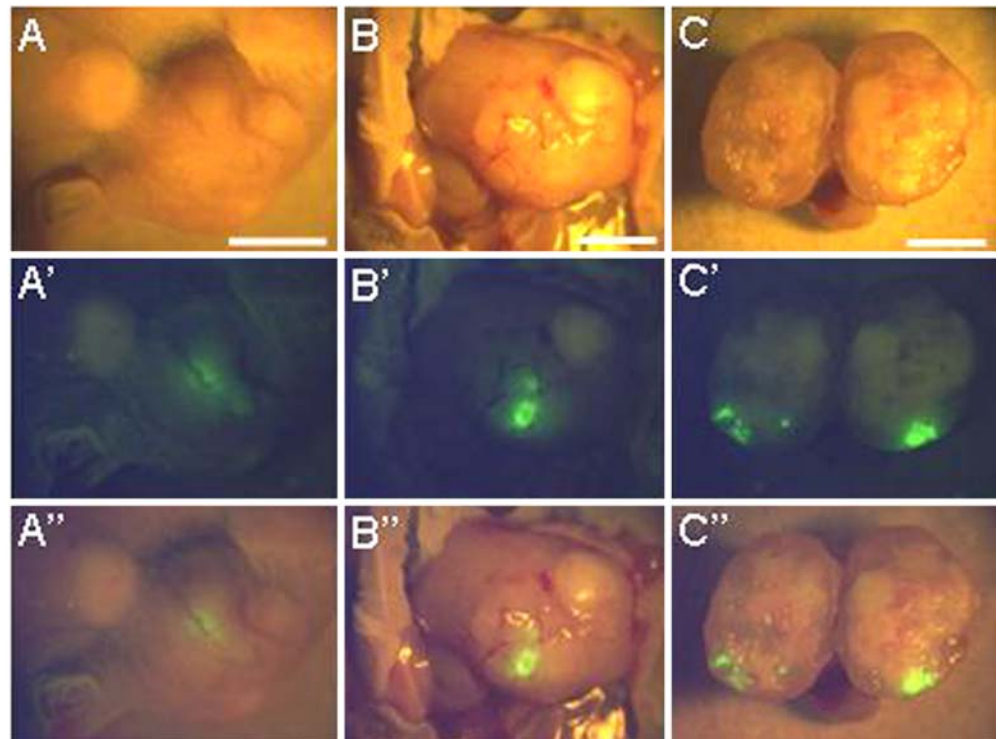
The tropism and migration of mammalian cells is well documented in the literature, particularly cell migration during embryonic development and cell infiltration during wound healing. Furthermore, heavy infiltration of leukocytes is often seen in tumor tissues. Recent studies show directly that mammalian cells delivered systemically find their way into distant tumor tissues. It is described that neural stem cells injected i.v. in the mouse appear in an intracranial tumor [17]. Additionally, when the same cells are implanted intracranially at distant sites from the tumor, e.g. into the contralateral hemisphere of the brain or into the cerebral ventricles, the neural stem cells migrate through normal brain tissues to the vicinity of human glioblastoma tumors [17]. In another report, after infusion of ovarian cancer cells into the pleural space, these cancer cells migrate into established malignant pleural mesotheliomas [18]. Furthermore, it is also shown that i.v. injected tumor cells are able to preferentially colonize injury sites and healing wounds [95]. These authors suggest that increased macrophage infiltration may account for the preferential colonization of injured tissue sites.

The above studies indicate that mammalian cells systemically introduced into live animals are found at the sites of tumors. We therefore reasoned that it may be possible to develop tumor detection strategies using blood-borne labeled mammalian cells. Itoh et al. [96] have radio-labeled lymphokine-activated killer (LAK) cells cultured from peripheral mononuclear cells originating from human patients. Using a high resolution gamma camera, migration of i.v. injected LAK cells to the tumor site is monitored noninvasively in malignant brain tumors in patients.

Fig. 3 Visualization of C6 glioma and fibrosarcoma tumors with labeled mammalian cells. Nu⁻/nu⁻ mice bearing s.c. C6 glioma implant in the hind leg (*panel I, A-A'' to B-B''*) or human breast tumor implant under mammary fat pad (*panel II, A-A'' to C-C''*) were i.v. injected with GFP-labeled fibrosarcoma cells (5×10^5). Seven days after injection, fluorescent patches were seen only in the tumor regions of the animals. Exposure of the tumor tissues by reflection of the overlying skin and cross sections of the tumors revealed fluorescent patches were visible in all regions of the tumors without central or peripheral preference (*Bar=5 mm*).



II.



Such mammalian cell-mediated tumor detection strategy has also been recognized and confirmed in other laboratories [97, 98, 99]. Instead of radiolabeling, Kjaergaard et al. [100] use a fluorescent dye to label LAK cells before injection and trace their migration to tumors and to metas-

tases in animals. Here we report the imaging of tumors using light-emitting mammalian cells in animals bearing orthotopic MCF-7 human breast tumor implants or s.c. C6 rat glioma implants. A stable GFP-labeled HT1080 human fibrosarcoma cell line was established by transforma-

tion with retrovirus derived from an EGFP-encoding pLEIN plasmid construct (Clontech). We demonstrated that the i.v. injected GFP-labeled fibrosarcoma cells gained entry and replicated in established breast and glioma tumors and therefore allowed the real-time visualization of the solid tumors in nu⁻/nu⁻ mice (Fig. 3). Consistent with earlier studies in which nontumorous animals have been used as recipients for cell implantation [101], trace amounts of light-emitting fibrosarcoma cell clusters were also found scattered on the lung surface, while being completely absent in other organs. These results indicated that mammalian tumor cells display tumor-tropic properties, which does not appear to be tumor-implant dependent. The injected cells constitutively produced light-emitting proteins, and therefore when injected signaled the locations of tumors in live animals. Given the fact that stem cells also show tumor affinity, stem cells labeled with fluorescence or luminescence can be engineered and used for tumor detection. Such stem cell-mediated detection will provide a safer alternative to labeled tumor cells. Davidoff et al. [102] report that GFP-labeled bone marrow-derived cells, when injected into the tail vein, contribute to the endothelial cell lining of tumor neovasculature.

In addition to tumor detection, engineered mammalian cells, similar to light-emitting bacteria and viruses, have also been used to label wounds and monitor wound healing. Recently, Yang et al. [103] have used GFP-labeled fibroblasts to demonstrate cell tropism to wounds immediately after i.p. injection of cells. Therefore, it may be feasible to use light-emitting mammalian cells for detection of wounds and for monitoring of wound healing.

Advantages and disadvantages of using BVMC as probes for tumor imaging

Detection of tumors by optical imaging using GFP and luciferase provides a noninvasive methodology. The BVMC, when protected by the tumor immunoprivileged environment, replicate and amplify in the tumors, and therefore the signal-to-noise ratio is enhanced without the need of delivering a large amount of the probes. In addition, not only genes encoding light-emitting proteins but also genes encoding therapeutic proteins for tumor therapy can be delivered systemically. The replication capability also allows the delivery of therapeutic agents for long-term treatment without multiple injections. Long term intrinsic stability of the BVMC-based probes is not of concern. In contrast, in other tumor detection probes, such as radiolabeled antibodies, the half-lives of both the antibodies and the radioisotopes need to be carefully evaluated. Limitations associated with the light-emitting BVMC-mediated tumor detection strategy are limited penetration and the scattering of fluorescence and luminescence light and fluorescence-activating blue light through deep tissues. Therefore, in order to achieve light-based tumor visualization for humans, a further improvement in light detection resolution and image reconstruction is essential.

Conclusion

BVMC labeled with light-emitting proteins reveal the location of tumors and metastases in live animals. The same or similar live microorganisms may be used for tumor detection in the future in human patients. The GFP-based light-emitting system may also provide guidance for surgery to remove tumors that are marked by fluorescence. In addition to tumor detection, therapeutic agents, such as antitumor bacterial toxins, tumor suppressor proteins, angiogenesis inhibitors, and apoptotic regulators, in combination with tumor-detecting marker genes, may be delivered to tumors and metastases efficiently by BVMC for localized long-term cancer gene therapy.

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