The Generation of a Conditional Reporter That Enables Bioluminescence Imaging of Cre/loxP-Dependent Tumorigenesis in Mice

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Abstract

The ability to noninvasively quantify tumor burden from conditional (Cre/loxP-dependent) mouse cancer models would greatly increase their range of useful applications. We now report the generation of a reporter mouse that enables visualization of spontaneous tumor development from pre-existing conditional mouse tumor models via in vivo bioluminescence imaging. We demonstrate that bioluminescence can be “switched-on” in a Cre-dependent manner in every organ analyzed, and that this gives rise to a 4 and 6-log increase in light emission per mg of wet tissue weight. Furthermore, we highlight the utility of this reporter by showing that it can be used as a sensitive means to measure spontaneous Kras2v12-induced lung tumorigenesis in a pre-existing mouse model of non-small cell lung cancer. Taken together, our results suggest that this reporter may be combined with a wide-range of other Cre/loxP tumor mouse models, irrespective of their tissue specificity and render them immediately amenable to longitudinal monitoring of tumor growth and therapeutic response with a noninvasive in vivo imaging approach.

Introduction

The recent widespread application of Cre/loxP technology has resulted in a new generation of conditional mouse cancer models that can better recapitulate many salient features of human tumorigenesis (1). To date these models have proven most useful for furthering our understanding of in vivo tumor etiology (2, 3). However, like other spontaneous tumor mouse models (4–7), they also possess great potential to become an improved means with which to evaluate novel preclinical cancer treatments and to elucidate key genetic factors that underlie chemoresistance.

Despite comprising an improved tool for modeling cancer, the stochastic and often nonvisible nature of tumorigenesis associated with conditional tumor models has thus far limited their widespread application for such purposes. These characteristic features complicate attempts to correlate tumor burden with onset of treatment and conventional tumor latency-based estimates are prone to a high degree of error. Furthermore, as autopsy is often the most effective way to noninvasively determine cell viability in vivo to and to measure tumor cell kill in response to therapy (8). To image spontaneously arising tumor burden from existing Cre/loxP models via this approach, however, one needs a strategy to introduce tumor- or tissue-specific luciferase expression into the mouse model. To limit the need to generate, optimize, and validate novel independent transgenic luciferase mouse strains for each individual Cre/loxP model, we now report the generation of a ubiquitously expressing conditional luciferase reporter mouse that can be used to render a wide range of Cre/loxP mouse tumor models suitable for bioluminescence imaging.

Materials and Methods

Generation of the LucRep Mouse. The LucRep transgenic mouse strain was generated by pronuclear microinjection of the LucRep transgene (depicted in Fig. 1) into fertilized FVB/N oocytes as described previously (11).

Genotyping of transgenic mice was determined by PCR. The primer sequences and PCR reaction conditions to detect Luciferase (3), Cre (10), and Kras2v12 (12) alleles were as described previously.

Imaging Protocol. Bioluminescence was measured noninvasively using the IVIS imaging system (Xenogen Corp., Alameda, CA). All of the images were taken 10 min after i.p. injection of luciferin (225 mg/kg; Xenogen Corp.) as a 60-s acquisition, binning 10, unless otherwise stated in the text. During image acquisition, mice were sedated continuously via inhalation of 3% isoflurane (Abbott Laboratories Ltd., Kent, United Kingdom). Ex vivo bioluminescence imaging of isolated organs was performed immediately after euthanasia of the animals by CO2, 10 min after i.p. injection of luciferin as described. Dissected organs were placed on a sheet of black plastic and imaged by IVIS; strong bioluminescent signals remained detectable >30 min after dissection. Image analysis and bioluminescent quantification was performed using Living Image software (Xenogen Corp.).

Induction of Lung Tumors. Approximately 106 or 107 particles of AdCre were introduced directly into the lungs of compound LucRep/conditional Kras2v12 mice via intratracheal intubation as described previously (12).

Results

Because tumorigenesis is initiated by either Cre-mediated “knock-out” or “knock-in” of tumor-associated gene function in conditional (Cre/loxP-dependent) cancer models, we reasoned that the generation of a generally expressed conditional luciferase transgene would give rise to tumor-specific light emission after introduction into pre-existing conditional tumor models. Accordingly, we generated a small series of transgenic founders via pronuclear microinjection of the conditional luciferase reporter (LucRep) construct as depicted in Fig. 1.

To determine the likely utility of these transgenic founders, we crossed each one with a ubiquitously expressing Cre transgenic line (ACM-Cre; Ref. 14) and measured the extent of bioluminescence...
from the resultant F1 generation by IVIS (Xenogen Corp.). This approach clearly demonstrated that one transgenic line, hereby designated as the LucRep line, was capable of Cre-dependent luciferase expression (Fig. 2A). Southern analysis on DNA derived from the tails of LucRep and LucRep/ACM-Cre compound mice showed that the nonrecombined LucRep allele had integrated as a long concatamer (>20 copies) at one locus, which was subsequently reduced to single copy after Cre mediated recombination (data not shown).

We next measured the difference in bioluminescence from individual “switched” and “nonswitched” tissues by sacrificing and dissecting three LucRep-only and three LucRep/ACM-Cre compound mice after administration of luciferin. The amount of light emitted from each exposed organ was then quantified by IVIS.

This experiment showed that bioluminescence could be “switched on” in a Cre-dependent manner in every LucRep organ analyzed and gave rise to between a 4 and 6-log increase in light emission per mg of wet tissue weight (Fig. 2B). Furthermore, the extent of light emission from switched LucRep tissue was comparable with that of another bioluminescent mouse tumor model (Pomc05) that we have used previously to noninvasively determine in vivo tumor growth dynamics and response to therapy from a deep tissue site (10).

The amount of background luminescence arising from individual nonrecombined LucRep organs was essentially negligible in all of the cases except for the heart, liver, and spleen, which were seen to emit very low levels of light. The extent of this background luminescence is unlikely to significantly affect the sensitivity of most LucRep imaging applications, however, as light emission was only weakly detectable, mainly around the thorax of intact mice, at sensitive image acquisition settings (60 s, binning 10). We next crossed the LucRep mouse with a conditional oncogenic Kras2 transgenic mouse to demonstrate that the LucRep allele could be used to image spontaneous tumor development in vivo. We have shown previously that multiple NSCLC-like lung adenocarcinomas develop in conditional Kras2V12 mice after adenoviral-mediated delivery of Cre recombinase (AdCre) to the lungs (12). Accordingly, several compound LucRep/conditional Kras2V12 mice were imaged by IVIS at weekly intervals after the introduction of ~109 AdCre particles into their lungs via intubation.

The result of this experiment showed that the LucRep allele could enable noninvasive visualization of conditional Kras2V12-induced lung tumorigenesis by IVIS (Fig. 3A). Direct imaging and histological processing of the lungs from this mouse additionally emphasized the sensitivity of this imaging approach, because the light detected from the mouse depicted in Fig. 3A was shown to originate from a single lesion measuring between 1 and 2 mm in diameter (see Fig. 3, B and C).

Longitudinal tumor growth from several AdCre-infected LucRep/conditional Kras2V12 compound mice was also followed over time, after the introduction of ~107 particles of AdCre to their lungs (Fig. 4, A and B). Measured bioluminescence arose slightly faster in this experimental cohort, and ex vivo analysis of the lungs indicated the presence of as many as 40 independent lesions of varying size. Ex vivo analysis of these lungs also indicated that the majority of lung tumors induced in this model should be suitable for bioluminescence imaging, because we observed only one macroscopically visible lesion from a total of 20 distinct lesions that did not glow.

The stochastic nature of spontaneous tumor onset associated with this mouse model of NSCLC is clearly illustrated by the graph depicted in Fig. 4B and, thus, highlights the usefulness afforded by noninvasive quantification, especially for the purposes of standardizing tumor burden within experimental cohorts of mice. More animals will need to be included in this experiment before a definitive statement can be made regarding the individual growth kinetics of Kras2V12-induced lung adenocarcinomas. However, there is little doubt that the LucRep allele will facilitate this work in the future.

Discussion

To date, with one exception (10), the advantages associated with noninvasive bioluminescence imaging of tumor burden in mice have been confined to xenograft-based models. The ability to noninvasively quantify tumor burden in living conditional tumor model mice, however, will ultimately lead to the development of more accurate models of human cancer that are better suited for evaluating and optimizing preclinical cancer therapy.

A similar conditional reporter transgene strategy to ours has been used previously, and because of the ubiquitous and conditional nature of reporter transgene expression, now comprises the most commonly used approach by the research community to test and validate novel Cre transgenic strategies (13). Because this reporter mouse is lacZ-based, however, its utility is effectively limited to studies on tissues postmortem. We now demonstrate that a ubiquitously expressed conditional luciferase transgene can be used as a sensitive means to noninvasively visualize cells that have undergone Cre-mediated recombination in living mice. We also show that this reporter can be used to noninvasively follow tumor growth over time via sequential measurement of tumor cell light emission, which illustrates that expression of the reporter is stable and that it can be used effectively to measure tumor burden from deep tissue sites like the lung. Furthermore, because of the ubiquitous nature of conditional luciferase transgene expression, we propose that the LucRep mouse will enable longitudinal analyses of spontaneous tumor development and response to therapy for a wide variety of conditional tumor models in the future.

A condition that must be met for such a purpose is that all of the conditional alleles present in a particular cell are switched, resulting in cells that carry one or more oncogenic lesions together with an activated luciferase reporter. This criterion can be met under two circumstances. First, when the luciferase reporter switches more efficiently than the conditional alleles that initiate tumor growth upon recombination. Second, when conditions are created in which all of the conditional alleles switch efficiently in the cell.

In the first situation, luciferase will also switch on in some cells in...
which no oncogenic lesions have been induced. Because these cells do not clonally expand, however, tumor growth can still be monitored effectively, as they will not provide an appreciable contribution to signal. We have not tested whether the LucRep reporter switches more effectively than the various conditional tumor suppressor and oncogene alleles. This issue is complicated by the fact that different alleles show large differences in Cre-mediated recombination (14), making the utility of the reporter dependent on the alleles with which it is combined. Switching efficiency is also dependent on the expression level of Cre recombinase in the cell. Therefore, in conditions where partial switching of conditional oncogenic lesions occur, the frequency of resultant tumors suitable for bioluminescence imaging will need to be determined empirically. We have found that these issues also apply to the Rosa26-LacZ reporter, which switches less well than several conditional alleles studied by us (14).

The second situation requires a relatively high level of Cre recombinase in the cell, which can be achieved using somatic gene transfer with vectors that deliver high transient expression of Cre.
recombinase. We have found that AdCre is particularly suited for this purpose, as the majority of infected cells appear to switch all of the floxed alleles (12). Our data are in agreement with this, because to date we have observed only one macroscopically visible tumor \textit{ex vivo} without concomitant luciferase expression. This implies that in the context of our mouse model of NSCLC, >90% of \textit{Kras2}^{12/12}-induced tumors should be amenable to noninvasive imaging via the LucRep allele. In more generalized terms, we believe that the LucRep reporter mouse will be most useful for imaging conditional tumorigenesis when Cre expression is introduced via the germ line in a tissue/cell type restricted manner or when introduced somatically via adenoviral or lentiviral expression vectors. It should be noted, however, that the extent of background bioluminescence may become significant when LucRep is used in conjunction with conditional tumor models with low tumor penetrance (\textit{i.e.}, in situations where many more normal cells switch relative to resultant tumorigenesis). If considered in terms of wet weight, a background of 1 mg switched normal tissue would be unlikely to affect the visualization of a 10-mg tumor, whereas 500 mg of switched normal tissue would. This issue did not apply to our model of NSCLC, because we switch only a very small fraction of the cells. As a result we observed no appreciable difference in bioluminescence before or several weeks after AdCre infection. Currently the LucRep reporter mouse lacks the spatial resolution of the Rosa26-LacZ reporter, because an antibody has yet to be developed against luciferase that can be used for immunohistochemical detection in paraffin-embedded tissue. One can envisage, however, that the future development of such an antibody will additionally increase the value of the LucRep mouse as a reporter, because it will then enable the detection of Cre recombinant cells in both living and fixed tissues.

References


Fig. 4. Longitudinal measurement of \textit{Kras2}^{12/12}-induced lung tumor growth from individual mice. \textbf{A}, three sequential images taken at 2-week intervals of mouse 36948; a representative mouse developing multiple \textit{Kras2}^{12/12}-induced lung tumors (images taken 10 min after i.p. administration of luciferin; 60 s acquisition, binning 10). The number of weeks annotating each individual image refers to the time post-AdCre infection. \textbf{B}, graph depicting the longitudinal measurements of \textit{Kras2}^{12/12}-induced lung tumor growth from individual mice. Light emission is expressed as corrected relative light units (\textit{i.e.}, measured light post-AdCre infection minus measured light pre-AdCre infection).
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