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Imaging Primary Lung Cancers in Mice to Study Radiation Biology

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Abstract

Purpose—To image a genetically engineered mouse model of non-small cell lung cancer with micro-CT to measure tumor response to radiation therapy.

Methods and Materials—The Cre-loxP system was utilized to generate primary lung cancers in mice with mutation in K-ras alone or in combination with p53 mutation. Mice were serially imaged by micro-CT and tumor volumes were determined. A comparison of tumor volume by micro-CT and tumor histology was performed. Tumor response to radiation therapy (15.5 Gy) was assessed with micro-CT.

Results—The tumor volume measured with free-breathing micro-CT scans was greater than the volume calculated by histology. Nevertheless, this imaging approach demonstrated that lung cancers with mutant p53 grew more rapidly than lung tumors with wild-type p53 and also showed that radiation therapy increased the doubling time of p53 mutant lung cancers five-fold.

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Conflicts of Interest: none.

Conclusions—Micro-CT is an effective tool to noninvasively measure the growth of primary lung cancers in genetically engineered mice and assess tumor response to radiation therapy. This imaging approach will be useful to study the radiation biology of lung cancer.

Keywords

Genetically Engineered Mouse Models of Cancer; Lung Cancer; Micro-CT; K-Ras; p53

Introduction

Lung cancer remains the leading cause of cancer death in the United States (1). The most common subtype of lung cancer is non-small cell lung cancer (NSCLC), which accounts for approximately 85% of all lung cancer diagnoses (2). Despite advances in radiation treatment delivery and the routine use of concurrent chemo-radiotherapy, many NSCLCs are not locally controlled and most patients with NSCLC die from their disease. In order to improve the outcome of NSCLC with radiation therapy, investigators have carried out valuable studies in radiation biology using different pre-clinical systems.

Traditional preclinical systems to study radiation biology include *in vitro* cell culture and xenograft mouse models (3). In xenograft models, limited numbers of human tumor cell lines are injected into immune-compromised mice, such as severe combined immunodeficient (SCID) or nude mice. Although this approach utilizes human cancer cells, the mouse tumor stroma may not be optimized to interact with human cancer cells, so these xenograft models may fail to recapitulate complex tumor–stroma interactions (4), which may be important in tumor response to radiation therapy (5). Moreover, defects in DNA repair, which are characteristic of some strains of immunodeficient mice (6), may alter the response of tumor stroma to radiation therapy and complicate the analysis of tumor response. Another potential limitation of a system that relies on immunodeficient mice is the challenge of assessing the role of the immune system in response to radiation therapy.

Autochthonous or primary mouse tumors have been studied less frequently. In this system, spontaneous cancers develop in tumor-prone strains of mice (7,8). This approach circumvents the limitations of tumor-stroma mismatch and host immunodeficiency that are inherent to xenograft systems. However, this system is challenging for radiation biology experiments because the anatomic location of each spontaneous tumor will vary from mouse to mouse. Although pieces of a spontaneous tumor can be propagated at a defined anatomic site in syngeneic mice to potentially facilitate radiation biology experiments, the growth rate of murine tumors may accelerate with *in vivo* passages (8).

Alternative model systems that utilize primary mouse tumors are genetically engineered mouse models (GEMMs) of human cancer (4). These tumors develop within a native tumor stroma in a mouse with an intact immune system. Moreover, tumors develop in a temporally- and spatially-restricted manner, which can facilitate radiation therapy. Although these tumors do not consist of human cancer cells, the gene mutations that initiate tumorigenesis in the mouse are in many cases identical to mutant oncogenes and tumor suppressor genes in human cancer. Moreover, in these models, “conditional” gene mutations have been engineered into the mouse germline at the endogenous gene locus, so that after Cre-mediated recombination the mutant gene is expressed at physiological levels from the endogenous promoter.

For example, we have developed a mouse model of NSCLC, which is initiated by activation of oncogenic K-ras (9) and mutation of p53 (10). K-ras and p53 are commonly mutated in human NSCLC (11). This GEMM not only recapitulates human NSCLC at the histological level (10), but also by gene expression (12). Here, we utilize this GEMM of NSCLC to serially

image lung cancers with micro-CT to compare growth rates among models and to quantitate the effects of radiation therapy. We demonstrate that whole lung radiation therapy can safely be delivered to cause tumor growth delay and thereby establish this GEMM as a new model to study radiation biology.

Methods and Materials

Generation of primary lung cancers and tissue processing

Lung tumors in *LSL-K-ras*^{G12D}, *LSL-K-ras*^{G12D} *p53*^{Fl/Fl}, and *LSL-K-ras*^{G12D} *p53*^{R270H/Fl} mice were generated as previously described (10). All procedures with animals in this study were approved by both the Institutional Animal Care and Use Committee at Massachusetts Institute of Technology and the Subcommittee on Research Animal Care at Massachusetts General Hospital.

Radiation Treatment

Mice were immobilized and treated with 15.5 Gy whole lung irradiation as described in Supplementary Figure 1. This dose was selected because it is similar to current fraction sizes of radiosurgery for lung cancer (13, 14).

Micro-CT Scans

CT data were acquired on a combined high-resolution single-photon emission CT (SPECT) scanner (Gamma-Medica X-SPECT, Northridge, CA) using 50 kVp X-rays with 500-mA current. Radiation dose to the mouse with the micro-CT scan was 22 cGy per scan according to TLD measurements. Secondary multiplanar and 3D reconstructions and tumor volumes were calculated with Amira (TGS, San Diego). This approach requires approximately 75 minutes per mouse: Micro-CT image acquisition 10 minutes/mouse, reconstructions 5 minutes/mouse, and tumor contouring and volume calculation approximately 60 minutes/mouse depending on the number of tumors contoured. Tumor margins were identified by contrast thresholding, which allows the tumor (soft tissue density) to be seen against the surrounding lung (air density). In some cases where the tumor is adjacent to another mass of soft tissue density, such as the heart, the radiologist used his best clinical judgment to determine the margin of the tumor.

Histological measurement of tumor size

Tumor areas were determined using Bioquant Image Analysis software in manual measurement mode. Volumes were calculated from histological sections by integrating the tumor area for each section over the distance between contiguous sections (typically every 100 microns).

Results

With the goal of employing a GEMM of human lung cancer to study radiation biology, we utilized the Cre-loxP system to generate primary lung cancers in mice. After inhalation of Adeno-Cre, *LSL-K-ras*^{G12D} mice developed low grade lung tumors that expressed oncogenic K-ras, while *LSL-K-ras*^{G12D}; *p53*^{Fl/Fl} and *LSL-K-ras*^{G12D}; *p53*^{R270H/Fl} mice developed more aggressive adenocarcinomas (10), which expressed oncogenic K-ras and no or R270H mutant p53 (Supplementary Figure 2). Lung tumor growth was monitored by serial micro-CT scans with 74 micron resolution. Analysis of micro-CT scans of a *LSL-K-ras*^{G12D} mouse following Adeno-Cre infection demonstrated that micro-CT imaging can be used to identify lung tumors and monitor tumor growth over time in this model (Figure 1A and Supplemental Movie 1). When serial sections of the lungs from this mouse were examined by histology, tumors identified on the micro-CT correlated with individual tumors within the mouse lungs (Figure

1B and Supplemental Movies 2A and 2B). Tumor volumes in the *LSL-K-ras*^{G12D} mouse were calculated from the serial micro-CT data sets using Amira software. At the initial micro-CT scan 12 weeks after Adeno-Cre infection, differences in tumor size were evident (Figure 1C). Tumor volumes calculated by micro-CT at 6 months after Adeno-Cre infection, strongly correlated ($r^2=0.95$) with volumes calculated by histological analysis (Figure 1D). However, the volume calculated by micro-CT consistently overestimated the size of tumors compared to the volume determined by histology (Figure 1D). The increased volume calculated by micro-CT may reflect both an integrated volume resulting from respiratory motion of the lung tumors as well as tumor shrinkage during tissue fixation and processing. Indeed, when we compared the maximal tumor dimensions by micro-CT with histology, we observed that the maximal tumor dimensions measured by micro-CT exceeded the maximal tumor dimensions measured by histology in all three dimensions (Supplementary Figure 3).

Having successfully used micro-CT to measure lung tumor growth in a *LSL-K-ras*^{G12D} mouse, we next utilized micro-CT to compare the growth rate of lung tumors in *LSL-K-ras*^{G12D} and *LSL-K-ras*^{G12D}; *p53*^{F1/F1} mice. Most lung tumors in *LSL-K-ras*^{G12D} mice are adenomas or low-grade adenocarcinomas (Figure 2A). In contrast, lung tumors in *LSL-K-ras*^{G12D}; *p53*^{F1/F1} mice, which have deleted the tumor suppressor p53, are higher-grade adenocarcinomas (Figure 2B). Consistent with the known difference in aggressiveness between lung tumors from *LSL-K-ras*^{G12D} and *LSL-K-ras*^{G12D}; *p53*^{F1/F1} mice (10), serial imaging of lung tumors with micro-CT demonstrated more rapid tumor growth in lung cancers lacking p53 (Figure 2C, $p=0.003$).

Finally, we utilized micro-CT to image lung cancers with and without radiation therapy in *LSL-K-ras*^{G12D}; *p53*^{R270H/F1} mice. After Adeno-Cre infection, lung cancers develop in these compound conditional mutant mice that express R270H mutant p53, which is analogous to the hot-spot R273H mutant p53 found in human lung cancer (11). In the absence of radiation therapy, the lung cancers grew rapidly (Figure 3A) with a doubling time of approximately 11 days (Figure 3C). After a baseline micro-CT scan, littermates were treated with 15.5 Gy whole lung radiation therapy. Follow-up micro-CT demonstrated marked tumor growth delay (Figure 3B) as the doubling time increased approximately 5-fold ($p=0.01$).

Discussion

We have previously described a GEMM of NSCLC (9,10). In this study, primary lung cancers from this mouse model were serially imaged by micro-CT to measure tumor growth and response to radiation therapy. Micro-CT is superior to MRI in imaging lung tumors due to the better contrast between the air and the soft tissue, whose interface can cause artifacts in MRI. Using micro-CT, we have been able to detect lung tumors below 1 mm³. One of the strengths of this serial imaging approach is that the radiation response of individual tumors can be followed. Because tumor size can vary at the start of an experiment (Figure 1), therapeutic studies with this model that do not utilize pre-treatment imaging will require a relatively large number of mice to account for inter-tumor heterogeneity. Another strength of this model is that tumor initiation occurs by Cre recombinase. Therefore, by crossing the *LSL-K-ras*^{G12D}; *p53*^{R270H/F1} mice with the growing list of other mice carrying Cre-activated mutant alleles, the role of these genes in the response of lung cancer to radiation therapy can be tested. Moreover, our imaging approach can be used to study the response of individual tumors to multi-fraction radiation regimens.

Our approach to imaging lung cancers with micro-CT also has some potential limitations. First, our method of measuring tumor volume may be effected by respiratory and cardiac motion because we did not image the mice with either respiratory or cardiac gating. Second, each micro-CT scan exposes the mice to a dose of radiation (22 cGy). Finally, we have observed

that pleural effusions or atelectasis occur in a minority of the mice, which can limit the identification of the lung cancer.

Despite these potential limitations, we have successfully used micro-CT to image a mouse model of primary NSCLC and have shown that this approach can measure tumor response to radiation therapy. This model system will be useful to study the radiation biology of lung cancer and to test novel radiation sensitizers in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Micro-CT detects the growth of primary lung cancers in mice. A. Axial images and 3D representations of tumor volumes from a micro-CT of a *LSL-K-ras*^{G12D} mouse 4, 5, and 6 months following Adeno-Cre infection. The heart is labeled in the image on the right and individual tumors are numbered 1 through 8. B. Hematoxylin and eosin stained axial section of the lungs from the same mouse imaged in A cut at a similar level as the micro-CT image identifies tumors numbered 1 through 8 by histology. C. Tumor volumes of 4 different lung tumors from a *LSL-K-ras*^{G12D} mouse imaged serially over time. Not all tumors are included as smaller tumors were not visible at earlier time points. X-axis indicates weeks following Adeno-Cre infection. D. Tumor volumes measured by micro-CT (y-axis) compared to the volume of the same tumors measured by histology (x-axis).

Figure 2.

Micro-CT imaging demonstrates that lung cancers lacking p53 grow more quickly than lung tumors with wild-type p53. A. Hematoxylin and eosin stained section of a low-grade lung tumor with wild-type p53 in a *LSL-K-ras*^{G12D} mouse. B. Hematoxylin and eosin stained section of a high-grade lung adenocarcinoma in a *LSL-K-ras*^{G12D}; *p53*^{FL/FL} mouse. Scale bars, 50 μm. C. Relative tumor volume measured by micro-CT of lung cancers from *LSL-K-ras*^{G12D}; *p53*^{FL/FL} and *LSL-K-ras*^{G12D} mice. A total of 7 tumors in *LSL-K-ras*^{G12D}; *p53*^{FL/FL} mice and 7 tumors in *LSL-K-ras*^{G12D} mice were contoured (p=0.003, 2 tailed t-test of the comparison of slopes). Micro-CT scans were completed 14 and 28 days after the baseline micro-CT scan. Error bars represent the S.E.M.

Figure 3.

Micro-CT imaging detects lung cancer response to radiation therapy in *LSL-K-ras*^{G12D}; *p53*^{R270H/F1} mice. A. Representative axial images of a *LSL-K-ras*^{G12D}; *p53*^{R270H/F1} mouse not treated with radiation therapy demonstrating rapid growth of a lung cancer (red arrow) after 1 month (right). B. Representative axial images of a *LSL-K-ras*^{G12D}; *p53*^{R270H/F1} mouse before (left) and one month after 15.5 Gy whole lung radiation therapy demonstrating slow growth of a lung cancer (red arrow). C. Doubling time in days of lung cancers in *LSL-K-ras*^{G12D}; *p53*^{R270H/F1} mice with (+) and without (-) 15.5 Gy whole lung radiation therapy ($p=0.01$, 2-tailed unpaired t-test). A total of 8 tumors without treatment and 7 tumors with radiation therapy were analyzed. Error bars represent the S.E.M.