Detection of Primary Lung Tumors in Rodents by Magnetic Resonance Imaging

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ABSTRACT

This report describes recent efforts to develop and apply small animal magnetic resonance imaging methods to the study of lung tumors in mice. Magnetic resonance (MR) images obtained with respiratory gating, with data collection synchronized with the respiration of the animal, allow visualization of submillimeter tumors in animals treated with a lung carcinogen. Comparison of the MR images with gross pathology of these lungs demonstrates the utility of the imaging methods for measuring tumor burden. As a noninvasive imaging modality that uses nonionizing radiation, MR is well suited to longitudinal studies aimed at understanding the factors that control the onset and development of lung tumors and their response to therapy in a wide variety of animal models.

INTRODUCTION

Lung cancer is the leading cause of cancer death in men and women in the United States (1). Molecular changes in proto-oncogene and tumor suppressor genes have been detected in all stages of lung carcinogenesis, with inactivations of the p53, Ink4a/Arf, and wild-type Kras2 being the most common genetic alterations observed in human lung cancers (2). Revolutions in molecular biology and genomics have led to the development of many new rodent models of disease, and many of the known genetic alterations commonly found in both mouse and human lung tumors suggest that there are similarities in the development of lung cancer in rodents and humans at the molecular level (3). With animal models widely used in both basic and preclinical sciences, findings ways to conduct animal experiments more accurately and efficiently becomes a key factor in the success and timeliness of research. Magnetic resonance imaging (MRI) is a powerful imaging modality for characterizing animal systems and animal models of disease. As a noninvasive, nondestructive technique, in vivo MRI permits a wide variety of longitudinal studies not possible with other destructive analytical methods. Although the opportunities for characterization are significant, lungs present unique challenges for MRI (4), requiring the development of new and innovative methods. A review of small animal MRI of lungs has appeared recently (5). Among the complicating factors for the study of lungs by 1H magnetic resonance (MR) methods are the following: (a) low tissue density and low water content within the lung severely limits signal-to-noise; (b) variations in magnetic susceptibility associated with the many air-tissue interfaces of the alveoli and bronchioles result in short T2* and T2 relaxation times; and (c) respiratory and cardiac motions lead to significant image blurring in the absence of motion-synchronized data acquisition. In this study, we demonstrate that respiratory-gated 1H MRI can detect submillimeter lung lesions in mice treated with the carcinogen, benzo(a)pyrene. The ability to noninvasively detect lung tumors at an early stage of disease will enable a wide variety of studies to provide insight into the factors that influence the onset and progression of lung cancer and its therapeutic response.

MATERIALS AND METHODS

MRI. Respiratory-gated, spin-echo MR images of mice were collected in an Oxford Instruments (Oxford, United Kingdom) 4.7 tesla, 40-cm bore magnet. The magnet is equipped with Magnex Scientific (Oxford, United Kingdom) actively shielded, high-performance (10 cm inner diameter, 60 G/cm, 100-μs rise-time) gradient coils and is interfaced with a Varian NMR Systems (Palo Alto, CA) INOVA console. All data were collected using a Stark Contrast (Erlangen, Germany) 2.5-cm birdcage radiofrequency coil. Before the imaging experiments, mice were anesthetized with isoflurane and were maintained on isoflurane/O2 (1–1.5% v/v) throughout data collection. Animal core body temperature was maintained at 37 ± 1°C by circulation of warm air through the bore of the magnet. During the imaging experiments, the respiration rates for all mice were regular and ∼2 s−1. Synchronization of MR data collection with animal respiration was achieved with a home-built respiratory-gating unit (6), and all images were collected during postexpiratory periods. Imaging parameters are repetition time (TR) = 3 s, echo time (TE) = 20 ms, 2.5 cm FOV (field of view), and slice thickness = 0.5 mm.

Lung Tumorigenesis Studies. All animal procedures were performed in accordance with guidelines of Washington University’s Animal Studies Committee. A lung tumor bioassay was conducted using 6-week-old wild-type (p53+/−Ink4a/Arf+/−) and p53−/−Ink4a/Arf−/− transgenic mice on the A/J mouse background (7, 8). Mice were given a single i.p. injection of benzo(a)pyrene (100 mg/kg body weight) in 0.1 ml of tricaprylin and were terminated six months after exposure to the carcinogen. During the bioassay, all of the animals were observed daily for clinical signs of ill health and weighed individually twice a month for the duration of the study. At termination, all of the animals were euthanized by CO2 asphyxiation after MRI imaging. The lungs were fixed in Tellyesniczky’s [90% ethanol (70% v/v), 5% glacial acetic acid, and 5% formalin (10% v/v buffered formalin)] overnight and then stored in 70% ethanol for evaluation of number and size of the tumors before paraffin embedding for histopathology. Tumors on the surface of the lungs were examined under a dissecting microscope to determine the number of tumors present and to measure the diameter of each tumor.

RESULTS AND DISCUSSION

As noted in the Introduction, lungs present several unique challenges to study by MRI. However, the very factors that make it difficult to image healthy lung parenchyma, including low tissue density, low water content, and variations in magnetic susceptibility within the lung, aid in the detection of tumors by increasing the contrast between healthy and pathological tissue. Fig. 1 (top) shows a series of nine contiguous coronal, respiratory-gated spin-echo images of a transgenic mouse (with alterations in both p53 and Ink4a/Arf) 6 months after treatment with a tobacco-specific carcinogen benzo(a)pyrene. An expanded view of the highlighted center slice is shown in Fig. 2 (bottom, left). Under the selected experimental conditions, the images of healthy mouse lung parenchyma are completely black (Fig. 1, bottom), with signals attributable to the heart and its major blood vessels suppressed because of flow effects and cardiac motion (because electrocardiograph gating was not used in this study). The bright spots visible in the images of Fig. 1 (top) are, therefore, attributable to lung tumors. The largest of these tumors is approximately 2 mm in diameter; the smallest is <0.6 mm in diameter. Fig. 2 (top, left) shows a single slice from a series of coronal images of...
pulmonary lesions from each of three stages of disease (hyperplasia, adenoma, and adenocarcinoma) were analyzed, and tumor diameters were recorded. Fig. 3 shows light photomicrographs of these three stages of lung lesions, and their histopathological characteristics are described here. The low-power micrograph of hyperplasia shows that the sepal architecture is largely maintained, and the lesion is focal and not well demarcated. Under high-power magnification, cells show little atypia, and their shape is often cuboidal. Features of adenoma cells seen under low power include a papillary pattern and some compression of adjacent parenchyma. Under high power, monomorphic, generally well-differentiated cells, supported by fibrovascular stroma, are observed. Lung adenocarcinoma displays a highly infiltrative character in the low-power micrograph and exhibits a marked cellular atypia and pleomorphism when examined under high magnification. Fig. 3G, a plot correlating tumor progression and pulmonary-lesion size after fixation, shows a strong association of tumor diameter with histological typing of either adenomas or adenocarcinomas. Logistic analysis using a generalized linear model shows significant association ($P < 0.01$) with an odds ratio of $1.6 \pm 0.5/0.1$-mm diameter. The carcinoma frequency was significantly increased among tumors larger than 2.0 mm in diameter. This finding shows that the image resolution and detection limits demonstrated in this study are well matched to the size of typical malignant lung lesions in mice.

The results presented in this paper demonstrate that MRI is a powerful imaging modality for the in vivo characterization of lung tumors in mice. The data clearly show that MRI can detect submillimeter tumors in mouse lung. Because MRI is noninvasive and nondestructive and uses nonionizing radiation, it is an ideal method for longitudinal studies of tumor development and therapeutic response. With the wide array of

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**Fig. 1.** Contiguous coronal slices, ventral to dorsal, from a series of respiratory-gated, spin-echo magnetic resonance (MR) images of a $p53^{+/+}$ Ink4a/Arf$^{+/+}$ mouse (top) 6 months after treatment with benzo(a)pyrene and of a control mouse (bottom). Images were collected with TR = 3 s, TE = 20 ms, FOV = 2.5 cm, slice thickness = 0.5 mm, and 128 x 128 data matrix, four averages. The white scale bars on these images indicate a length of 5 mm. As demonstrated in the bottom panel, the images of healthy mouse lung parenchyma collected under these experimental conditions are completely black and the bright spots visible in the images in the top panel are attributable to lung tumors. Fig. 2 (bottom, left) shows an expanded view of the center slice (indicated by the white box) from the top panel of images.

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a mouse having much more extensive lung-tumor development. Large tumors are seen in the right lung of this mouse and fill nearly its entire left lung. After imaging experiments, this mouse was sacrificed and its lungs harvested. Fig. 2 (top, right) shows a photograph of these lungs, and the red arrows highlight the correspondence between the tumors visible in the MR image and those in the photograph. Fig. 2 (bottom, right) is the corresponding photograph of the lungs of the mouse whose MR image is shown in Fig. 2 (bottom, left). Although the orientation of the lungs in this photograph is different in this image, the overall correspondence between the tumors seen in the photograph and those shown in the MR image is excellent. Recently, Fisher, et al. (9) reported the use of MRI to confirm the appearance and regression of lung tumors in mice. Herein we report the in vivo detection of primary lung tumors at a submillimeter level, correlated with histopathology, in mice.

As shown in Fig. 2, pulmonary lesions seen under a conventional dissecting microscope are detectable by MR imaging and can be quantified through 3D reconstruction. This is highly significant because the size of a given lung tumor closely correlates with tumor progression. Fig. 3 demonstrates this correlation between the size of the lesion and the degree of tumor progression. Ten
transgenic mice now available, we are confident that MRI will provide important insights into the factors that control the onset and development of tumors and their response to therapy.

REFERENCES
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