NEOPLASIA
www.neoplasia.com

Volume 20 Number 10 October 2018 pp. 975–984 975

A Novel Method for Quantifying Total Thoracic Tumor Burden in Mice[1]

Pavitra Viswanath[*†], Shaohua Peng[*], Ratnakar Singh[*2], Charles Kingsley[‡], Peter A. Balter[§] and Faye M. Johnson[*†]

[*]Department of Thoracic Head & Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX; [†]The University of Texas Graduate School of Biomedical Sciences, Houston, TX; [‡]Department of Imaging Physics, The University of Texas MD Anderson Cancer Center, Houston, TX; [§]Department of Radiation Physics, The University of Texas MD Anderson Cancer Center, Houston, TX

Abstract

Mouse models are powerful tools to study lung cancer initiation and progression *in vivo* and have contributed significantly to recent advances in therapy. Using micro-computed tomography to monitor and study parenchymal and extra-parenchymal metastases in existing murine models of lung cancer is challenging owing to a lack of radiographic contrast and difficulty in achieving respiratory gating. To facilitate the analysis of these *in vivo* imaging studies and study of tumor progression in murine models we developed a novel, rapid, semi-automated method of calculating thoracic tumor burden from computed tomography images. This method, in which commercially available software is used to calculate the mass of the thoracic cavity (MTC), takes into account the aggregate tumor burden in the thoracic cavity. The present study showed that in tumor-free mice, the MTC does not change over time and is not affected by breathing, whereas in tumor-bearing mice, the increase in the MTC is a measure of tumor mass that correlates well with tumor burden measured by lung weight. Tumor burden calculated with our MTC method correlated with that measured by lung weight as well as or better than that calculated using four established methods. To test this method, we assessed metastatic tumor development and response to a pharmacologic PLK1 inhibitor in an orthotopic xenograft mouse model. PLK1 inhibition significantly inhibited tumor growth. Our results demonstrate that the MTC method can be used to study dynamic changes in tumor growth and response to therapeutics in genetically engineered mouse models and orthotopic xenograft mouse models of lung cancer.

Neoplasia (2018) 20, 975–984

Abbreviations: DICOM, Digital Imaging and Communications in Medicine; GEMM, genetically engineered mouse model; KP, KRAS TP53; micro-CT, micro–computed tomography; MTC, mass of the thoracic cavity; PLK1, polo-like kinase 1; RECIST, Response Evaluation Criteria in Solid Tumors; ROI, region of interest; SCP, sum of cross-product; T&V, tumor and vessel volume.

Address all correspondence to: Faye M. Johnson, Department of Thoracic Head & Neck Medical Oncology, Unit 432, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA.
E-mail:; fmjohns@mdanderson.org

[1] Funding: MD Anderson's animal facilities are supported by the National Institutes of Health through MD Anderson's Cancer Center Support Grant (P30CA016672). This work was supported by individual donations (FMJ) and by the Office of the Assistant Secretary of Defense for Health Affairs through the Lung Cancer Research Program (award no. W81XWH-17-1-0206). Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense.

[2] Present address: Department of Comparative Biosciences, University of Illinois Urbana-Champaign, Champaign, Illinois.

Received 26 June 2018; Accepted 2 August 2018

1476-5586

Introduction

Lung cancer is the second most common cancer in men and women, with about 234,030 American adults being diagnosed with the disease in 2018 alone. Despite advances in its treatment, lung cancer remains the leading cause of cancer death [1]; patients have a five-year survival rate of less than 15%. The development of better lung cancer therapies requires a deeper understanding of the molecular signaling mechanisms that drive the disease's formation, maintenance, and progression. Cancer disease progression studies are very important in assessing response-guided treatment strategies in patients with solid tumors as they allow for clinical trial analysis to calculate time-to-event endpoints and more importantly, assist in determining clinical treatment response and/or failure.

In vivo studies in murine models play important roles in identifying the mechanisms of lung tumorigenesis and assessing the safety and efficacy of novel drug therapies. The models most commonly used to study lung tumorigenesis and therapeutic strategies are subcutaneous xenograft models created by implanting human cell lines or patient tissues into immunocompromised mice [2]. However, murine orthotopic models of lung cancer, although technically more challenging than subcutaneous models, have a tumor microenvironment more representative of that in humans, making them better suited for the study of disease progression *in vivo* [3]. Orthotopic models of lung cancer can be generated in immunocompromised or immunocompetent mice by intrabronchial injections [4], intrathoracic injections [5], tail vein injections that result in lung metastasis [6], or percutaneous injections of lung cancer cells into the left lung [7]. Most genetically engineered mouse models (GEMMs) have the advantages of being orthotopic and having an intact immune system. Many GEMMs encompass several mutations found in non–small cell lung cancer (NSCLC), including KRAS, BRAF, EGFR, LKB1, TP53, and NFκB [8]. Most GEMMs of NSCLC are adenocarcinoma models and one of the most commonly used models was established by engineering a Lox-Stop-Lox conditional KRAS G12D mutation in the endogenous KRAS locus [9,10]. Combining KRAS activation with the concomitant inactivation of p53 results in more aggressive tumors that also metastasize. The relationship between primary tumor nodules and individual metastases could be established in studies in which KRAS activation and p53 inactivation are achieved by infecting the mouse lung with lentiviral Cre [11].

The evaluation of lung cancer progression in the lungs of mice is primarily based on end-stage procedures performed after necropsy, such as histopathologically analyzing lung tissue, weighing the lungs, or counting lung tumors. Although these *ex vivo* procedures offer many opportunities to perform molecular and cellular analyses, they are limited to only one measurement and do not provide details about the dynamic processes that occur over time *in vivo*. In contrast, micro–computed tomography (micro-CT) can be used to noninvasively study the dynamic changes of tumor progression in preclinical models [12]. Although micro-CT is technically challenging because of respiratory movement artifacts, it provides visual and quantitative information about the whole lung in a three-dimensional manner with high resolution and sensitivity. More importantly, micro-CT allows for the longitudinal assessment of therapeutic interventions in different treatment groups as well as that of the extent of disease in individual mice. In addition, despite delivering a relatively large radiation dose per acquisition, micro-CT is safe, causing no radiotoxicity to the lungs of mice undergoing weekly micro-CT for up to 12 weeks [13]. However, unlike CT in humans, micro-CT in mice does not include the use of radiographic contrast, and achieving respiratory gating with micro-CT in mice is difficult.

Micro-CT has been successfully used to detect lung tumors and evaluate lung tumor burden in many NSCLC mouse models [14–16]. Methods previously used to quantify metastatic tumor burden relied on Response Evaluation Criteria in Solid Tumors (RECIST)-like assessment, in which the maximal tumor diameter and largest perpendicular diameter were measured in the coronal plane and the tumor burden was calculated from the sum of the cross products [17]. However, such methods may have reader bias, and variability has been observed among readers. In addition, these methods measure only the largest tumors and ignore small tumors that, in aggregate, may contribute significantly to the total tumor burden. Other methods that have been used to quantify tumor burden include tumor nodule segmentation [18]; segmentation of the aerated lung volume with respiratory gating [19]; manual segmentation of the chest space [16]; tracking of individual nodules [20]; modeling of tumors as ellipsoids [21]; and volumetric measurement of the combined tumor and vasculature from a threshold-based region growing algorithm with manual and semi-automated segmentation [22]. However, these methods are labor-intensive, require specific skill sets in radiology, and do not take all thoracic metastases into consideration. Murine micro-CT imaging has limitations that are not present for human CT imaging. As the murine micro-CT imaging is not respiratory gated, the probability of air escaping into the lungs and variance in the breathing period is high despite breath hold at the time of imaging. Also, tumor tissue and vasculature cannot be distinguished in the non-contrast micro-CT imaging as they have similar X-ray densities.

Therefore, we developed a semi-automated, unbiased method of analyzing micro-CT without respiratory gating for the *in vivo* quantitative assessment of lung tumor mass in mice. We propose this imaging technique and tumor mass analysis as a quantitative tool that is reliable and yields dynamic information on disease progression. Given the basic assumption that the aerated mass of the thoracic cavity (MTC) in an adult mouse does not change over time unless disease progresses, our imaging and analysis protocol is designed to permit comparisons between different groups, enable the evaluation of individual animals over time, and provide specific information about disease progression and metastasis. Unlike aerated lung volume, which is dependent upon the respiratory phase of breath hold, MTC is dependent on the differences in density between tumors and parenchymal tissue independent of respiratory state. In the present study, we evaluated our model by comparing its performance with that of with four established methods and a reference method in assessing tumor burden in a murine orthotopic model of metastatic lung cancer.

Materials and Methods

Cells

Highly metastatic 344SQ (miR200-expressing) cells were derived from transgenic mice with KRAS (G12D) TP53 (R172H) mutations (KP mice) [23] and were a generous gift from Dr. Don Gibbons. The cells were maintained in RPMI 1640 with 10% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37°C as described previously [24].

Mice

All animal experiments were conducted in accordance with the laws of the United States and the regulations of the U.S. Department of Agriculture. All animal experiments were reviewed and approved