Early Lung Adenocarcinoma in Mice: Micro-Computed Tomography Manifestations and Correlation with Pathology

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Abstract

Lung cancer is the most common fatal malignancy for both men and women and adenocarcinoma is the most common histologic type. Early diagnosis of lung cancer can significantly improve the survival rate of patients. This study aimed to investigate the micro-computed tomography (micro-CT) manifestations of early lung adenocarcinoma (LAC) in mice and to provide a new perspective for early clinical diagnosis. Early LAC models in 10 mice were established by subcutaneously injecting 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) solution. Micro-CT scan and multiple planar reconstruction (MPR) were used for mouse lungs. Micro-CT features of early LAC, especially the relationships between tumor and bronchus, were analyzed and correlated with pathology. Micro-CT findings of early LAC were divided into three types: non-solid (n = 8, 6%), partly solid (n = 85, 64%) and totally solid (n = 39, 30%). Tumor-bronchus relationships, which could be observed in 110 of 132 (83%) LAC, were classified into four patterns: type I (n = 16, 15%), bronchus was truncated at the margin of the tumor; type II (n = 33, 30%), bronchus penetrated into the tumor with tapered narrowing and interruption; type III (n = 38, 35%), bronchus penetrated into the tumor with a patent and intact lumen; type IV (n = 99, 90%), bronchus ran at the border of the tumor with an intact or compressed lumen. Micro-CT manifestations of early LAC correlated well with pathological findings. Micro-CT can clearly demonstrate the features of mouse early LAC and bronchus-tumor relationships, and can also provide a new tool and perspective for the study of early LAC.

Introduction

Lung cancer remains the leading incidence of malignant tumor, accounting for male and female cancer mortality rates of 28% and 26%, respectively [1,2]. CT screening for lung cancer has been shown to obviously reduce lung cancer mortality in a series of recent randomized controlled trials [3–7]. Consequently, low-dose computed tomography (CT) has been widely used for lung cancer screening and has detected a large number of small or micro lung nodules that pose a diagnostic challenge to radiologists. Among the lung nodules found by screening CT in a high risk population, 1.1% to 12% of them were malignant and most of them were lung adenocarcinomas (LAC) [8–11]. However, these early LAC lack characteristic CT findings, such as lobulation, speculation and contrast-enhancement. Therefore, it is difficult to make a correct diagnosis for early LAC [12,13]. Furthermore, most of the tumors were too small to obtain a complete tissue sample after the intraoperative frozen section diagnosis, which led to a restricted CT-pathology correlation. Micro-computed tomography (micro-CT) of lung cancer in animal models can make up for the limitations of clinical study. In contrast to clinical CT, micro-CT possesses micron-sized resolution, which can show anatomical micro-structure information [14,15]. Enabled by the inherent contrast between air and tissue, micro-CT is a powerful modality for lung imaging and can clearly show the characteristics of...
early mouse LAC and also provide a novel viewpoint and tool for the
study of LAC.

Based on the pre-established mouse model of early LAC [16], this
study aimed to investigate micro-CT manifestations of early LAC and
tumor-bronchus relationship to afford new information for clinical
CT diagnosis.

Materials and Methods

Establishment of Mouse Model and Animal Preparation

The study was approved by the institutional review board of
Jinshan Hospital, Fudan University. Every effort was made to
minimize suffering and the number of animals used in each
experiment.

As previously described [16], early LAC models were established in
10 female KM mice (Jie Si Jie Laboratory Animal Company,
Shanghai, China) by subcutaneously injecting 0.2 ml
1-methyl-3-nitro-1-nitrosoguanidine (MNNG) solution (2.0 mg/ml)
(Ru Ji Biotech Company, Shanghai, China) once weekly for 4 weeks. At
the 100th day after the first injection, animals were anesthetized by
intraperitoneal injection of 0.02 ml ketamine solution (10 mg/ml).
The trachea was exposed via a midline neck incision. A 50% dose of silica
solution 0.4 ml was administered through the inferior vena cava to
induce pulmonary embolism and consequent deep breathing. The
trachea was ligated rapidly at the end of deep inspiration to maintain
lung inflation. The mice were euthanized by cervical dislocation and
immersed in 10% formalin solution for 24 h.

Micro-CT Scanning and Imaging Analysis

Mice chests were scanned by micro-CT equipment (Siemens in
vivo micro-CT) with a field of view 24 mm × 24 mm. According to
the pre-experimental results, acquisition parameters were chosen as
follows: data acquisition method bin1, resolution 19.0 μm, voltage
80 kV, current 500 μA, time exposure 600*3 ms, scanning time
64 min and magnification 5.0. Multiple planar reconstruction (MPR)
of lung images in three orthogonal planes was performed with the
reconstruction algorithm dsf2 and matrix size 2048*2048 by using a
post-processing workstation (Inveon research workstation). Three
lung lobes of each mouse were randomly selected and the number,
diameter, margin and solid component of tumors were analyzed and
recorded in transverse, sagittal and coronal reconstructed images. The
interior or exterior bronchi of tumor and the tumor-bronchus
relationships were observed emphatically. The spatial relationships
between bronchus and tumor were defined according to the following
methods: (1) central bronchus: bronchus located within the area of
central 1/2 radius of tumor; (2) peripheral bronchus: bronchus
located in the area of peripheral 1/2 radius of tumor; (3) exterior
bronchus: bronchus bordered tumor. The sequential imaging sections
that were identical with the pathological sections were reconstructed
in 37 focused tumors, by referring the tumor’s pathological shape and
its adjacent large vessels and pleura.

Histopathologic Analysis

The lung lobe of each mouse was separated, grossly observed for
nodules, and fixed in neutral formalin. The 3-mm-block included the
nodule that was sampled and embedded in paraffin. The whole
paraffin block was cut into a series of 3-μm sections, with an interval
of 100 μm. Sections were stained with hematoxylin and eosin (H&E)
and were microscopically analyzed to determine the histologic type, in
addition to the size, shape, margin, growth pattern and bronchus of
the tumor. Thirty-seven tumors were selected to strictly correlate
between the micro-CT findings and pathology, focusing on the
tumor-bronchus relationship.

Statistical Analysis

Statistical analyses were performed using SPSS 20.0 statistical
software (SPSS Inc., Chicago, IL, USA). A Pearson chi-square test was
conducted to compare the relationships both between tumor size and
bronchial position in groups of partly solid and totally solid tumors,
and between tumor size and tumor-bronchus relationship patterns. A
P-value of less than .05 was considered to be a statistically significant
difference.
Results

Micro-CT Manifestations of Tumors

All 10 mice had tumor formation. The number of tumors ranged from 9 to 47, with a total of 231 tumors found by micro-CT. Three lobes were randomly selected from each mouse, and a total of 132 tumors were investigated. All the tumors were LAC that were confirmed by histology. The tumor size ranged from 0.1 mm to 1.8 mm, with a mean diameter of 0.56 mm.

Micro-CT findings of LAC were classified into three types based on the proportion of the solid component: non-solid (n = 8, 6%), partly solid (n = 85, 64%) and totally solid (n = 39, 30%). A non-solid tumor appeared as a well-defined, irregular ground-glass opacity with cribriform or tube-like lucent structures and spicular margin (Figure 1). A partly solid tumor appeared as a well-defined, irregular nodule with a central solid, peripheral ground-glass opacity and spicular margin. There were cribriform or tube-like low density structures in the peripheral ground-glass opacity (Figure 2). A totally solid tumor was a homogeneous solid density nodule with a smooth margin (Figure 3).

Figure 2. Micro-CT and photomicrograph (HE ×100) of a partly solid tumor. Micro-CT (A) shows that a partly solid nodule (white box) appears as a central solid with a homogeneous high density (black arrow); peripheral ground-glass opacity (white arrow) with cribriform and tube-like structures. Photomicrograph (B) shows a mixed growth pattern tumor. The center and periphery of the tumor appear as hilic (black arrow) and lepidic (white arrow) growth, respectively. There are intact alveoli and alveolar ducts in the periphery of the tumor that correlate with the cribriform or tube-like structures on micro-CT.

Figure 3. Micro-CT and photomicrograph (HE ×100) of a totally solid tumor. Micro-CT (A) shows a homogeneous solid nodule with a high density, smooth margin and some little round lucent structures (white box). Photomicrograph (B) shows a hilic growth tumor without intratumoural air-contained alveoli and patent bronchioli. There are some air-contained alveoli and patent bronchioli in the border of the tumor that correlate with little round lucent structures on micro-CT.
**Tumor-Bronchus Relationship**

One hundred ten (83%) out of 132 tumors had a direct relation with bronchus. The tumor-bronchus relationships were classified into four types as follows: type I (n = 16, 15%), bronchus was truncated at the margin of the tumor (Figure 4); type II (n = 33, 30%), bronchus penetrated into the tumor with tapered narrowing and interruption (Figure 5); type III (n = 38, 35%), bronchus penetrated into the tumor with a patent and intact lumen (Figure 6); and type IV (n = 99, 90%), bronchus ran at the border of the tumor with an intact or compressed lumen (Figure 7). With increased tumor size, the prevalence of type I tumor-bronchus relationship increased, whereas types II and III decreased, and type IV was mostly observed in medium-sized tumors. There were significant differences in the prevalence of tumor-bronchus patterns among different tumor sizes ($P = .006$). The relationship between tumor size and tumor-bronchus pattern is listed in Table 1.

Non-solid tumors were frequently less than 0.5 mm in diameter. The bronchus appeared as little round or tube-like lucent structures that penetrated into the center and the periphery of the tumor. The bronchus in partly solid and totally solid tumors were located at the center, periphery and exterior border of the tumor with an increasing prevalence. The bronchus could be found at the exterior border in almost all of partly solid tumors, whereas the prevalence of this occurrence was relatively less in totally solid tumors. The distributions of bronchus in partly solid and totally solid tumors are listed in

![Figure 4](image4.png)  
**Figure 4.** Type I tumor–bronchus relationship. Micro-CT (A) shows that a bronchus (white arrow) is obstructed abruptly by a totally solid tumor (white box). The slightly thickened-wall is seen in the bordering portion of the bronchus (white arrow). (B) Photomicrograph (HE ×100) of the same tumor shows an identical tumor–bronchus relationship with a slightly thickened-wall of the bronchus both inside and outside the tumor (black arrow).

![Figure 5](image5.png)  
**Figure 5.** Type II tumor–bronchus relationship. Micro-CT (A) shows that a bronchioli (white arrow) penetrates into an irregular partly solid tumor (white box) and interruption. Photomicrograph (HE ×100) of the same tumor (B) shows an identical tumor–bronchus relationship. The bronchioli (black arrow) and intratumoural alveolar ducts correlate with the tube-like and round lucent structures on micro-CT.
Table 2. There were significantly statistical differences in the prevalence of bronchus among different locations of partly solid and totally solid tumors ($P < .001, P = .003$).

Histopathological Findings

Histopathologically, the tumors demonstrated three types of growth patterns: lepidic, hilic and mixed, which corresponded to non-solid, totally solid and partly solid tumors on micro-CT. No tumor cells were found in all ventilating bronchioles. Varying envelop, compression and invasion were found in respiratory bronchioles. Tumor cells proliferated, accumulated and extended inside the alveolar septa, and compressed, diminished and obliterated the alveoli to varying degrees. The lepidic tumor evolved into the mixed and hilic tumors. On micro-CT, the heterogeneous ground-glass opacity and cribriform structures corresponded pathologically to the thickened neoplastic alveolar septa and alveoli, respectively; the little round or tube-like lucent structures inside the tumor were the residual alveoli, alveolar ducts or patent bronchioli. The lepidic tumor cells were either hindered by the bronchioli and bronchus or grew around them, or both, in the process of spreading along the alveolar septa, and thus formed a well-defined margin

Figure 6. Type III tumor–bronchus relationship. Micro-CT (A) shows a bronchiole (white arrow) run through a solid tumor (white box) with a patent and intact lumen. Photomicrograph (HE ×100) of the same tumor (B) show a corresponding bronchiole (black arrow) with a smooth lumen.

Figure 7. Type IV tumor–bronchus relationship. Micro-CT (A) shows a bronchiole (white arrow) runs at the border of a solid tumor (white box) with an intact lumen. Photomicrograph (HE ×100) of the same tumor (B) shows a corresponding bronchiole (black arrow) at the border of the tumor.

Table 1. The Correlation Between Tumor Size and Tumor–Bronchus Relationship

| Tumor–Bronchus Relationships | Total | Tumor Size (n = 110) | P-Value * |
|-----------------------------|-------|---------------------|----------|
|                             |       | 0–0.5 (45) | 0.5–1.0 (60) | 1.0–2.0 (5) |       |
| Type I                      | 16 (15%) | 4 (9%)  | 10 (17%)  | 2 (40%) | .008  |
| Type II                     | 33 (30%) | 19 (42%) | 13 (22%)  | 1 (20%) | .000  |
| Type III                    | 38 (35%) | 27 (60%) | 11 (18%)  | 0 (0%)  | .000  |
| Type IV                     | 99 (90%) | 41 (68%) | 54 (90%)  | 4 (80%) | .000  |

P-value * .000 .000 .000 .083 /

* Pearson χ² test.
and tumor-bronchus relationships [24]. Our study showed that CT, it can provide more information about small bronchi, bronchioli be thoroughly investigated.

tube, were clearly displayed, and tumor-bronchus relationships could demonstrate the presence of lung tumors and could not clearly show the internal structure of tumors. Because of the motion artifacts of the tumor and tumor-bronchus relationship. Previous studies [17–23] used micro-CT to investigate the vessel supply, angiogenesis and therapeutic response of mouse lung cancer, as well as airway changes in a mouse asthma model. However, it could only demonstrate the presence of lung tumors and could not clearly show the internal structure of tumors. Because of the motion artifacts caused by the respiration or cardiac impulse of mice, it was difficult to obtain clear images in vivo [23]. Furthermore, the spatial resolution in previous studies was not high enough to obtain a satisfactory image. In this study, a state-of-the-art micro-CT scanner, with a 19-μm high resolution, and sacrificed mice were used to conduct a lung scan. In this way, the airways, from the biggest trachea to the smallest alveolar tube, were clearly displayed, and tumor-bronchus relationships could be thoroughly investigated.

Since the resolution of micro-CT is far superior to that of clinical CT, it can provide more information about small bronchi, bronchioli and tumor-bronchus relationships [24]. Our study showed that micro-CT display multiple tumor relevant bronchi in 78% of tumors and multiple types of tumor-bronchus relationship in 64% of tumors. The results suggested that the tumor-bronchus relationship was a prevalent CT sign, which was applied to investigate the occurrence and progression of tumors.

The Correlation Between Micro-CT Findings and Pathological Growth Patterns of Tumors

Our study showed that micro-CT findings of mouse LAC could be divided into three categories: non-solid, partly solid, and totally solid nodules that pathologically corresponded to three growth patterns: lepidic, mixed and hiliar. Meanwhile, these three categories of micro-CT findings corresponded to pure ground-glass, mixed ground-glass and solid nodules on clinical CT [25,26]. Owing to its micron-size resolution capability, micro-CT identify the compressed and residual alveoli, alveolar ducts and bronchioli, which appeared as cribriform or tube-like lucent structures delineated by the thickened neoplastic alveolar septa in non-solid nodules and the periphery of partly solid nodules. It is well known that LAC cells originate from alveolar epithelia cells [27–29], as found in this study, proliferate and extend inside the alveolar septa, and compress and diminish the alveoli. Our study showed that lepidic tumor cells were either hindered by the bronchioli and bronchus or got around them, or both, in the process of spreading along the alveolar septa, and correspondingly formed a well-defined margin and lobulation, an irregular shape, or spiculation. As the tumor cells aggregated, the growth pattern transitioned from lepidic to hiliar and formed a partly solid or even solid nodule, which frequently squeezed and obliterated the alveoli and alveolar ducts, bronchioli, and bronchus. Therefore, as tumor size increased, few bronchi and bronchus were found at the center of the tumor. Alternatively, as the solid component increased, fewer bronchi and bronchus were found at the center periphery, and exterior border. In almost all partly solid tumors the bronchus was found at the exterior border, but the bronchus of totally solid tumors was less frequently found there.

Discussion

The Advantages of Micro-CT in Investigating Mouse Lung Cancer Model

In the present study, micro-CT, with a resolution of 19 μm, was used to investigate early LAC in mice. The results indicated that micro-CT could clearly demonstrate the morphological characteristics of the tumor and tumor-bronchus relationship. Previous studies [17–23] used micro-CT to investigate the vessel supply, angiogenesis and therapeutic response of mouse lung cancer, as well as airway changes in a mouse asthma model. However, it could only demonstrate the presence of lung tumors and could not clearly show the internal structure of tumors. Because of the motion artifacts caused by the respiration or cardiac impulse of mice, it was difficult to obtain clear images in vivo [23]. Furthermore, the spatial resolution in previous studies was not high enough to obtain a satisfactory image. In this study, a state-of-the-art micro-CT scanner, with a 19-μm high resolution, and sacrificed mice were used to conduct a lung scan. In this way, the airways, from the biggest trachea to the smallest alveolar tube, were clearly displayed, and tumor-bronchus relationships could be thoroughly investigated.

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The Relationship Between Early LAC and Bronchus in Mice

As a result of LAC originating from the airway epithelium, the tumor causes the spatial relationship with the bronchus. The development of the tumor gradually leads to changes in bronchial morphology. Previous clinical studies [30] have indicated that CT demonstrated five types of tumor-bronchus relationships in 86% malignant and 76% benign nodules of less than 3 cm in diameter. The types are as follows: type I: bronchus was obstructed abruptly by the tumor; type II: bronchus penetrated into the tumor with tapered narrowing and interruption; type III: bronchial lumen shown within the tumor was patent and intact; types IV and V: bronchus ran around the periphery of the tumor with either intact lumen or displaced, compressed, and narrowed lumen. Malignant nodules had 59% of type I, 15% of type II, 15% of type III, 26% of type IV and 2% of type V. Types I, II, and IV were more common in malignant nodules than in benign nodules. The following studies obtained similar results [31–33]. In this study, similar manifestations were obtained by using micro-CT in mouse early LAC, and, thus, a similar classification was used. The first three types were the same as the clinical classification. Due to the small tumors and, consequently, little compression on border bronchus, type IV and type V could not be distinguished on micro-CT images. Thus, we merged type IV and type V into type IV; i.e., bronchi ran around the periphery of the tumor with an intact or compressed lumen.

Compared with that of previous studies, this study showed that the prevalence of each tumor-bronchus relationship was significantly different [29–32]. There were significantly fewer type I and more type IV in our micro-CT study. The mean diameter of tumors was only 0.56 mm, which was approximately equivalent to 11 mm of human lung tumors [16]. Small tumors lightly compressed border bronchioli, leading to less of type I and more of type IV tumor-bronchus relationship. As tumor size increased, the prevalence of type I increased and that of both type II and type III decreased. Furthermore, owing to the high resolution of 19 μm, micro-CT could display terminal bronchioli and alveolar ducts, and, thus, more subtle tumor-bronchus relationships could be observed. In previous clinical studies, most tumors were larger solid tumors that exerted compression on border bronchioli and resulted in more type I and less type IV and type V tumor-bronchus relationships. In addition, the resolution of clinical CT was not high enough to show distal to seventh-order bronchi and led to less incidence of tumor-bronchus relationship.

Table 2. The Distributions of Bronchi in Partly Solid and Totally Solid Tumors

| Tumor Number | Bronchial Location | pValue* |
|--------------|-------------------|---------|
|              | Centre            | Periphery | Exterior |
| Partly solid (n = 85) | 18 (21%) | 41 (48%) | 80 (94%) | .000 |
| Totally solid (n = 39)  | 6 (15%)  | 16 (41%) | 20 (51%) | .003 |

* Pearson χ² test.
This study had some limitations. First, in order to avoid motion artifact and indistinct image, micro-CT scan was performed in a euthanized and fixed mouse model. Second, because the trachea was ligated at the end of deep inspiration, the diameter of the bronchus might be slightly larger. Third, due to the lack of pathological criteria for mouse early LAC, we arbitrarily assumed early LAC according to the tumor size [16].

Conclusion
Micro-CT can clearly demonstrate the imaging features of mouse early LAC and tumor-bronchus relationships. This technique provides both a new tool and a new perspective for the study of early LAC, affording new information for clinical CT diagnosis of early LAC.

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