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Micro-computed tomography images of lung adenocarcinoma: detection of lepidic growth patterns

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ABSTRACT

Micro-computed tomography (μCT) provides extremely high-resolution images of samples and can be employed as a non-destructive inspection tool. Using μCT, we can obtain images comparable with microscopic images. In this work, we have attempted to take high-resolution images of the human lung using μCT. Compared to clinical high-resolution computed tomography (HRCT) images of living body (in-vivo imaging), we can obtain extremely high-resolution images by μCT of ex-vivo tissues (resected lungs) as three-dimensional data. The purpose of this study was to distinguish between areas of normal lung and lung cancer by μCT images in order to study the feasibility of cancer diagnosis using this novel radiological image modality. Ten resected human lungs containing primary cancer were fixed by Heitzman's methods to obtain high-resolution μCT images. After fixation of the lung, images of the specimens were taken by μCT between January 2016 and November 2017. The imaging conditions were tube voltage: 90 kV and tube current: 110 μA. To compare details of images gained by conventional HRCT and μCT, we measured the thickness of the alveolar walls of the normal lung area and the cancer area of which alveoli might be replaced by tumor cells, and compared their appearance by means of histopathological images. All the nodules were diagnosed as adenocarcinoma. The median whole tumor size was 18 mm (9 mm–24 mm). Each specimen was clearly divided into areas of normal alveolar wall and of thickened alveolar wall on μCT 'visually'. Median thickness of alveolar walls of the normal lung was 0.037 mm (0.034 mm–0.048 mm), and that of the cancer area was 0.084 mm (0.074 mm–0.094 mm); there was a statistically significant difference between both thicknesses by Student's *t*-test ($P < 0.01$). The area of thickened alveolar walls on μCT corresponded well with the area of microscopically lepidic growth patterns of adenocarcinoma. We found that μCT images could be correctly divided by alveolar walls into normal lung area and lung cancer area. Further detailed investigations with regard to μCT are needed to make comparable histological diagnoses using μCT images with conventional microscopic methods of pathological diagnoses.

Keywords: Lung cancer, Computed tomography, Micro-computed tomography, Three dimensional reconstruction, Pathological diagnosis

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INTRODUCTION

Some lung adenocarcinomas exhibit areas of ground glass opacity (GGO) on high-resolution computed tomography (HRCT), reflecting a lepidic growth pattern of tumor cells microscopically.[1] Therefore, lung tumors that display areas of focal GGO on HRCT contain components of histological lepidic tumor growth.[2] Pathological lepidic growth components of those adenocarcinomas show thickened alveolar walls because of tumor growth along with alveolar walls, and the change of the alveoli reflects areas of GGO on HRCT.

Micro-computed tomography (μCT) provides extremely high-resolution images of samples and can be used as a non-destructive inspection tool. It allows us to obtain images comparable with microscopic images. In this study we have attempted to take high-resolution images of the human lung using μCT. Several researchers have reported that alveolar ducts and pulmonary alveoli could be identified on μCT images.[3-6] However, it was difficult to achieve a direct observation of cancer cells on μCT images even with high-quality images of the lung.

The aim of this study was to detect adenocarcinoma of the lung by μCT. It became possible to distinguish normal lung from adenocarcinomas with lepidic growth pattern on μCT images not only visually but also pathologically. Nevertheless, it was still impossible to make pathological diagnoses based on μCT images alone other than adenocarcinoma with lepidic growth pattern. Based on these facts, this study was pilot study that showed pathological diagnoses of lepidic growth pattern performed only by μCT images. To evaluate how clear those images were, we compared thickness of the alveolar walls between areas of the normal lung and lung adenocarcinoma with the lepidic growth pattern. This study was first report which investigated images of lung cancer using μCT We showed μCT images in which thickened alveolar walls could be observed in lung cancer areas. This finding could be a surrogate for imaging cancer cells in adenocarcinoma specimens.

MATERIALS AND METHODS

Lung samples

Resected lungs containing primary cancer from ten patients were enrolled in this study, which were diagnosed as adenocarcinoma with part-solid or non-solid nodules on preoperative HRCT images in Nagoya University Hospital between January 2016 and November 2017. All information on radiological and pathological variables was collected from the medical records. This study protocol was approved by the institutional review boards (UMIN000015930). Informed consent was obtained before surgery from each patient.

Tissue preparation: Fixation of the lung

Ten each resected lung including tumor parts were divided into two specimens, one for pathological diagnoses within the standard clinical workflow, and the other for taking images by μCT for the present study. The lung specimens were maintained and fixed to obtain high-resolution images by μCT. Each specimen was inflated with a pressure of 30 cm H2O.

Micro-computed tomography

After fixation of the lung, images of the specimens were taken by μCT (made by Shimadzu Co., inspeXio SMX-90CT Plus MICRO FOCUS X-RAY CT SYSTEM). The imaging conditions were tube voltage 90 kV and tube current 110 μA, imaged at a spatial resolution of 13 μm×13 μm×13 μm/voxel using Micro-CT scanners. As for pixel number was 1024×1024× (478 to 2150).

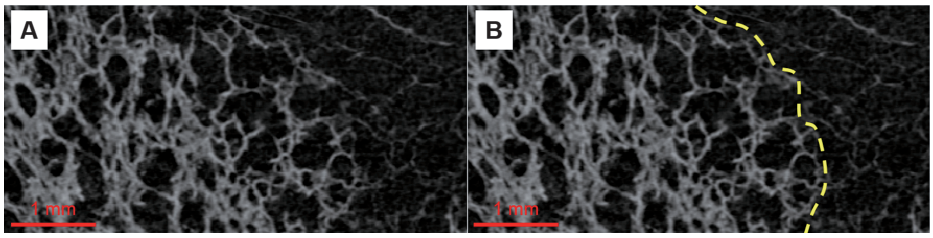

Fig. 1 These Images were taken by micro-computed tomography
Fig. 1A: This Image taken by μCT shows a part solid nodule. Thickened alveolar walls, and thin alveolar walls can be observed around the solid nodule. Image visualization was obtained by PLUTO software.
Fig. 1B: The image could be well divided into alveolar walls of normal and thickened areas (yellow line).

It took almost 20 minutes to take images for one specimen.

Definition of alveolar wall thickness

After conversion to DICOM form of μCT image data, image reconstruction was obtained by the PLUTO software[7] (Fig. 1A). Alveoli wall thickness was defined as the width of a white line on a μCT image (Fig. 1B). We measured alveolar wall thickness at 10 points in both the thin and thick areas on μCT for each specimen, and the average value of 10 points was defined as each thickness of the alveolar wall. To confirm whether or not those areas with thickened alveolar wall were consistent with pathologically lepidic growth pattern, we aligned two images gained by μCT and microscope using an especially developed registration method.[8-10]

Statistical analysis

Wilcoxon test was conducted in order to compare thickness of the normal alveolar wall and thickened alveolar wall on μCT images. All analysis was conducted using the JMP software program (version 13.0.0; SAS institute Inc., Cary, NC, USA). The results are expressed as the mean ± standard deviation and a *P*-value of less than 0.05 was considered statistically significant.

RESULTS

All the tumors were diagnosed as adenocarcinoma. The histological subtypes[11] were as follows: adenocarcinoma in situ (n=1), minimally invasive adenocarcinomas (n=2), invasive adenocarcinomas with lepidic-predominant (n=3) and invasive adenocarcinomas with papillary predominant (n=4). The median whole tumor size was 18 mm (9 mm–24 mm) with the median invasive tumor size was 10 mm (0 mm–19 mm). The area of GGO on HRCT could be seen clearly in the μCT image as a thickened alveolar wall area (Fig. 2-3).

The image could be clearly divided into thin and thickened alveolar wall areas. Median thickness of normal walls was 0.037 mm (0.034 mm–0.048 mm), and that of thickened walls was 0.084 mm (0.074 mm–0.094 mm). A significant difference was observed statistically in the thickness between thin and thickened alveolar walls ($P < 0.01$) (Fig. 4). Thickened alveolar wall areas on μCT corresponded well with the lepidic growth pattern microscopically (Fig. 5).

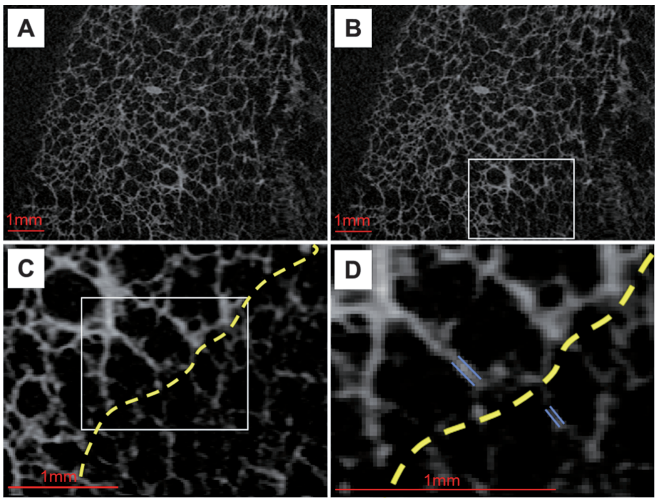

Fig. 2 These μCT images show images of lung adenocarcinoma case

Fig. 2A-B: This μCT image shows a non-solid nodule of adenocarcinoma case.

Fig. 2C: This image shows an enlarged view of enclosed area in Fig. 2B.

Fig. 2D: It could be well distinguished visually between normal alveolar walls and thickened alveolar walls. We measured the alveolar wall thickness (distance between blue lines) on μCT.

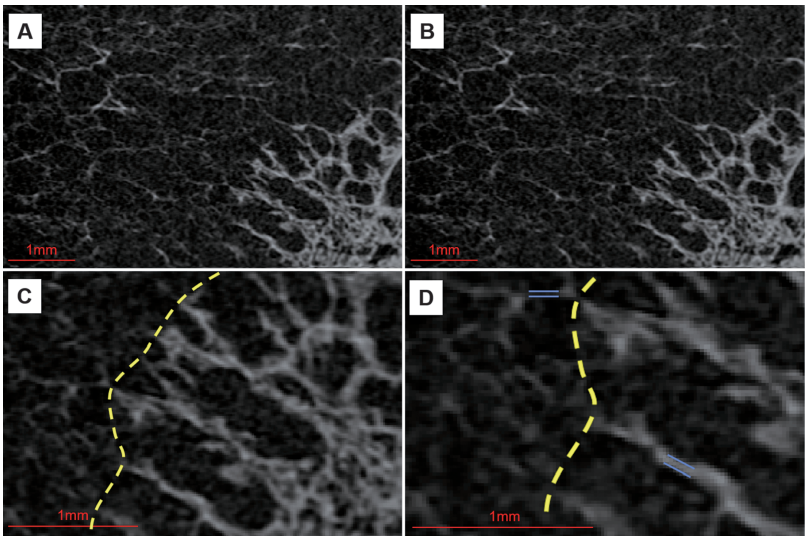

Fig. 3 These μCT images show images of lung adenocarcinoma case

Fig. 3A-B: μCT image shows part-solid nodule of adenocarcinoma case.

Fig. 3C: This image shows an enlarged view of the enclosed area in Fig. 3B.

Fig. 3D: Normal alveolar walls and thickened alveolar walls could be well distinguished visually. We measured the alveolar wall thickness (distance between blue lines) on μCT.

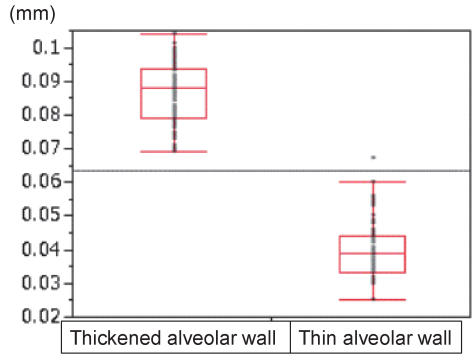

Fig. 4 A significant difference was observed statistically in the thickness between thin (0.039 ± 0.008 mm) and thickened alveolar walls (0.088 ± 0.009 mm) ($P < 0.01$)

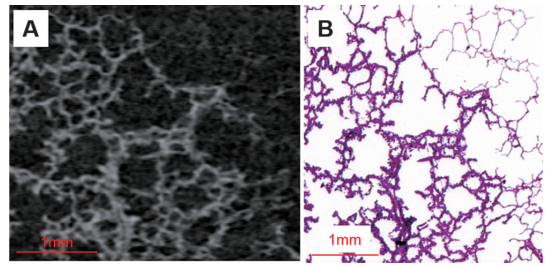

Fig. 5 Image registration of μCT (Fig. 5A) and microscope (Fig. 5B) image was performed in order to align the images of the same specimen
After spatial alignment, it could be confirmed whether or not thickened alveolar wall areas are consistent with the pathological lepidic growth pattern.

DISCUSSION

Clinical HRCT images were used for the diagnoses of the living body (in-vivo imaging). Any computational and diagnostic imaging analysis was constrained to the millimetre scale because of the limited resolution of the HRCT imaging technology. With this scale, although pulmonary vessels and lung segment could be observed, finer detailed anatomy was not observable compared to microscopic images. μCT, on the other hand, allows imaging of ex-vivo tissues at a resolution of tens of micrometres. Using this micrometer scale, the alveoli and small airways could be clearly observed.[3-6]

μCT was developed for industrial use as a non-destructive inspection tool and provides extremely high-resolution images of samples. The appearance of reconstructed μCT images obtained in this study is comparable with that of microscopic images. We have attempted to take high-resolution images of the human lung using μCT.[10] Several researchers reported their study taking pulmonary specimens by μCT. Mai et al investigated the process of lung fibrosis in patients with idiopathic pulmonary fibrosis (IPF) using explant lungs prior to transplants by μCT. Their findings supported the theory that alveolar collapse might be the initial trigger for the fibroproliferative process in patients with IPF. They found that μCT could act as the bridge between thin-section CT and histological analysis.[3] McDonough et al tried to determine whether there was a relationship between small-airway obstruction and emphysematous destruction in

COPD.[4] In their study, μCT was used to measure the extent of emphysema and the number of terminal bronchioles per millilitre of lung volume. They found that narrowing and disappearance of small conducting airways before the onset of emphysematous destruction could be explained by the increased peripheral airway resistance reported in COPD. In the same way, μCT has been used to reveal the etiology of pulmonary diseases. Our previous work also showed that μCT could act as a bridge between HRCT and microscopic findings.[12] On μCT images, the differences in the alveolar wall thickness between normal lung and lung cancer could be clearly observed. These differences corresponded well to the pathological findings: those areas with thin alveolar walls corresponded to the pathological area of normal lung. On the other hand, areas with thickened alveolar walls corresponded to pathological areas of lung adenocarcinoma with lepidic growth pattern. Our current results further support the notion that μCT could serve to bridge between HRCT and microscopic images.

We found that μCT images could be correctly divided as alveolar walls into normal lung areas and lung cancer areas on radiological images. Our study and other reports have pointed to micro-scale imaging as a new frontier in radiology. Further investigations are needed to make histological diagnoses using μCT images more accurate. Rapid three-dimensional imaging with μCT could well provide an advantage over conventional microscopic diagnoses which involves preparation of costly histopathological slides and much processing time. If comparable histopathological diagnoses of pulmonary nodules could be obtained by μCT within the living body, the need for lung cancer patients to be examined for pretreatment bronchoscopic or surgical lung biopsy could well be reduced in the future.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

DISCLOSURES

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