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Original Article

Photodynamic Diagnoses of Malignant Pleural Diseases Using the Autofluorescence Imaging System

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Background: We conducted a study on photodynamic diagnosis (PDD) using autofluorescence in video-assisted thoracic surgery for minute intrathoracic small dissemination or early malignant pleural mesothelioma.

Methods: Autofluorescence is the spontaneous emission of light that occurs when mitochondria, lysosomes, and other intracellular organelles absorb light. In normal tissues, green autofluorescence of approximately 520 nm is observed in response to 400–450 nm blue excitation rays. However, in cancer lesions, green autofluorescence is reduced due to thickening of the mucosal epithelium, a decrease in autofluorescent substances, etc., and the color spectrum thus shifts to red-violet. This phenomenon is the basis of PDD.

Results: The color spectrum shift was observed in all tumors located on the pleural surface but not in cases with pleural fibrous disease. Among patients with primary lung cancer, those with pleural infiltration (pl) scores of 1 or greater showed color spectrum shifts due to reduced autofluorescence.

Conclusion: Localization of pleural lesions by autofluorescence imaging was found to be useful. In primary lung cancer cases, differentiation between pl0 and pl1 lesions appears to be useful for determining therapeutic strategies including surgical procedures.

Keywords: photodynamic diagnosis (pdd), thoracic malignant disease, autofluorescence imaging system, malignant pleural mesothelioma, lung cancer

Introduction

For minute dissemination and early malignant pleural mesothelioma, which are considered to be factors favoring intrathoracic recurrence after surgery for lung cancer, diagnostic imaging and intraoperative visual inspection have limitations. Thus, more accurate assessment and diagnostic methods are needed. With a focus on autofluorescence, we conducted a study on the novel photodynamic diagnosis (PDD) system.

Autofluorescence is the spontaneous emission of light that occurs when mitochondria, lysosomes, and other intracellular organelles absorb light. The sources of autofluorescence in human tissue reportedly include nicotinamide-adenine dinucleotide phosphate, and flavin-adenine dinucleotide, as well as collagen and fibronectin.[1,2] In normal tissues, green autofluorescence of approximately 520 nm is observed in response to 400–450 nm blue excitation rays. However, in cancer lesions, green autofluorescence is reduced due to thickening of the mucosal epithelium, a decrease in autofluorescent substances, an increase in fluorescence absorbing substances, etc., and a shift in the fluorescence spectrum is thus observed (**Fig. 1**). Imaging and observation of this reduction in fluorescence and the wavelength shift are the principles

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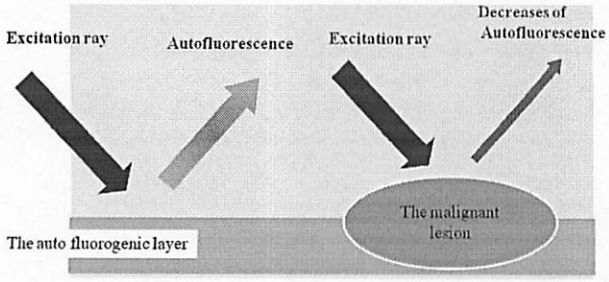

Fig. 1 The principle of the autofluorescence observation. Normal tissue: In response to blue excitation rays of approximately 400–450 nm, green autofluorescence of approximately 520 nm is observed. Malignant Lesion: Autofluorescence is reduced due to thickening of the mucosal epithelium, adecrease in autofluorescent substances, an increase in fluorescence absorbing substances, etc., causing the color spectrum of emitted fluorescence to shift.

underlying operation of the autofluorescence imaging system. In the 1990s, autofluorescence bronchoscopy using these principles was developed[3] and has since been further refined and applied to diagnosis in various fields.[4–6] This study aimed to establish a diagnostic method using a device composed of a thoracoscope (rigid scope) equipped with the autofluorescence imaging system for intrathoracic malignancy.

Materials and Methods

Among patients who underwent respiratory surgery using a thoracoscope during the period between February 2012 and February 2013, the thoracic cavity of those providing consent was observed with the autofluorescence imaging system. This study included a total of 31 patients, composed of 22 with primary lung cancer (including one patient with pleural dissemination), five with metastatic lung tumors, two with malignant pleural mesothelioma (both cases biopsy-confirmed), and two with benign tumors. Patient characteristics are shown in **Table 1**. A thoracoscope equipped with the autofluorescence imaging system was inserted through the thoracoscope insertion port (12 mm) to observe the thoracic cavity. The autofluorescence imaging system used in this study was developed by modifying the color fluorescence imaging system for endoscopy (PDS-2000, Hamamatsu Photonics, Shizuoka, Japan)[7,8] and is equipped with a small charge-coupled device (CCD) camera; our system allows simultaneous white light imaging and filtered autofluorescence imaging. The Olympus Endoscopic System (OES) attachment was used for color fluorescence photography

Table 1 The object cases of this study

- Primary lung cancer: 22
  Men:women 13:9
  Pathology: Adenocarcinoma 13
  Squamous cell carcinoma 8
  Pleomorphic carcinoma 1
  T factor: T1a/T1b/T2a/T2b/T3: 6/6/6/3/1
  pl factor: pl0/pl1/pl2: 18/1/3
  Grade: 1/2/3: 14/5/3
- Metastatic pulmonary tumor: 5
- Malignant pleural mesothelioma: 1
- Benign tumor: 2 (thymona, neurinoma)

and the camera was connected to a thoracoscope with a surgical drape attached. A xenon light source (300 W) capable of generating excitation rays of mainly 420–450 nm was used as the light source. This clinical study was approved by the Ethics Committee of the Asahikawa Medical University, and informed consent was obtained from each patient prior to surgery.

Results

Visualization

Even in lesions that showed an unclear color spectrum shift on white-light images and were difficult to visualize, tumor sites were visualized as violet to red-violet by the autofluorescence imaging camera, in contrast to the green autofluorescence of the surrounding normal tissues. Images obtained in patients with pleural dissemination (metastatic breast cancer and carcinomatous pleurisy) (**Fig. 2**), and pl2 lung cancer (**Fig. 3**).

Visualization of each lesion

1. The color spectrum shift was observable in all patients with metastatic lung tumors located on the pleural surface. Moreover, the color spectrum shift was observable in patients with dissemination or malignant pleural mesothelioma on the parietal pleural surface but was not seen in those with pleural fibrous disease (**Fig. 4**).

2. Among patients with lung cancer, all 4 with pl1 or greater lesions (n = 4) showed a color spectrum shift due to reduced autofluorescence. Although the color spectrum shift was observable in two of the patients (11.1%) with pl0 lesions (n = 18), both were diagnosed as having pl1 lesions before and during surgery, and the borders between the lesions and normal tissues were unclear. No differences in histological type or malignancy grade were noted.

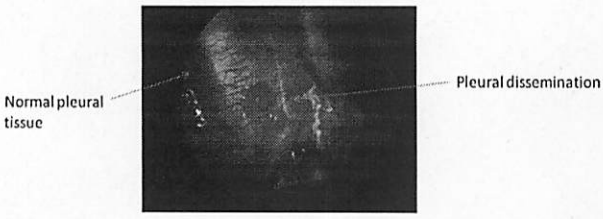

Fig. 2 Pleural dissemination (Metastatic breast cancer). The normal tissue emitted green autofluorescence, and the tumor lesion appeared blue-violet in the color spectrum.

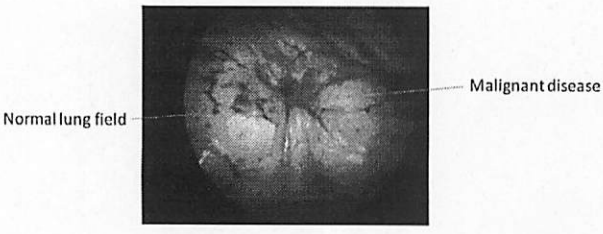

Fig. 3 Lung cancer (a case of pl2). A color spectrum shift consistent with that in an area showing infiltration was observed.

Discussion

Because a factor possibly contributing to early recurrence of lung cancer might be the presence of minute dissemination, accurate diagnosis of this pathological condition is an extremely important issue in planning therapeutic strategies. Moreover, early diagnosis of malignant pleural mesothelioma, which carries a poor prognosis due to the lack of effective therapies, has also attracted attention. Furthermore, preoperative diagnostic imaging or thoracoscopic visualization alone is well known to have limitations, and more accurate diagnostic methods are thus needed. With a focus on autofluorescence emitted by normal tissues in response to excitation rays, we examined whether diagnostic accuracy could be improved by applying PDD. At present, although diagnostic methods employing autofluorescence are widely performed with bronchoscopy and colonoscopy,[4–6] there are few reports on such methods using a thoracoscope for intrathoracic malignancy.[9] While applying the autofluorescence imaging system for bronchoscopy, we conducted a study on the diagnosis of intrathoracic malignancy using thoracoscopy with autofluorescence imaging. The color spectrum shift was observed in all tumors located on the pleural surface, and it did not admit false negative. But it was limited to the tumor on the pleural surface.

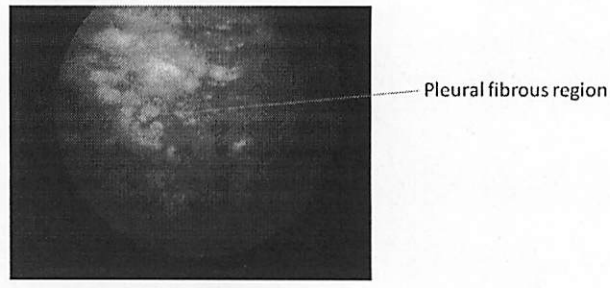

Fig. 4 Pleural fibrous disease. No color spectrum shift was observed in the pleural fibrous disease area.

Currently available fluorescence imaging systems include PDS-2000 (Hamamatsu Photonics, Hamamatsu, Japan),[7,8] which we currently use and have been modifying, SAFE-3000 (PENTAX, Tokyo, Japan),[10] and Autofluorescence Imaging (Olympus, Tokyo, Japan),[11] and each system has been demonstrated to be useful for the diagnosis of early bronchial carcinoma by bronchoscopy. In addition, the accuracy of autofluorescence imaging has been further improved by the use of not only various light sources but also a bronchoscope equipped with the narrow-band imaging system.[12] There is also a report describing an attempt to improve accuracy by the addition of red light.[13] The problems encountered in using a thoracoscope with autofluorescence imaging, as performed in the present study, involve diagnostic accuracy. These problems might be attributable to the device itself or to differences among lesions.

First, the problems with the device include low accuracy due to slightly darker and less clear images than those obtained with white-light imaging. While the performance of the CCD camera can contribute to reduced accuracy, we also consider the light source to be a noteworthy factor. In this study, the excitation wavelength used for irradiation was generated employing a 300 W xenon light source. Although the peak excitation ray is blue light at approximately 420–450 nm, spectral analysis showed peak wavelengths to vary from 400 to 480 nm. This may explain lesions appearing slightly bluish rather than red-violet. Moreover, it has been pointed out that the light intensity emitted by our device is rather low for optimal observation. At present, we are developing a device equipped with a light-emitting diode to increase light intensity which can emit rays with a constant excitation wavelength. In this study, we encountered no problems associated with the optical telescopes.

The problems attributable to properties of the lesions are related to the depth of diagnosable pleural infiltration.

Although color spectrum shift in lesions exposed on the pleura was captured by the autofluorescence imaging camera at a high frequency, the depth of pleural infiltration in patients with lung cancer can make it difficult to delineate the actual tumors. Reduced autofluorescence was visualized in patients with a lesion pathologically diagnosed as pl1 or greater, while there were many patients with pl0 lesions in whom the reduction could not be delineated. Because emphasis is placed on the pl factor in the histological classification of lung cancers, differentiation between pl0 and pl1 lesions has important implications for therapeutic strategies including surgical procedures. Thus, the results of this study provide potentially useful information. Moreover, differentiation between benign and malignant lesions is another important challenge. Because the malignant lesions observed in this study had an appearance clearly different from that of benign lesions, such as fibrotic hyperplasia and neurogenic tumors, differentiation from benign lesions is assumed to be possible to some extent. However, there is also a report describing standardized uptake values on positron emission tomography as being high in inflammatory masses, such as immunoglobulin G4-related disease.[14] Future studies are needed to refine the autofluorescence device and improve our technique.

Based on our present results, autofluorescence imaging alone has limitations in delineating the borders and properties of lesions. Thus, we are now studying 5-aminolevulinic acid (5-ALA), a photosensitizing substance. Orally administered 5-ALA is metabolized to protoporphyrin IX, a precursor of heme, in the mitochondria of cells and is retained within malignant cells; protoporphyrin IX emits red to pink light of approximately 630 nm.[15] We have thus launched another study aimed at improving the diagnostic accuracy of this system with preoperative oral administration of 5-ALA and intraoperative observation of the contrast in autofluorescence between malignant lesions and the surrounding tissues.

Conclusion

We examined intrathoracic malignancy diagnosis employing an autofluorescence imaging system. Using the principles of autofluorescence, which is reduced in malignant tumors, we were able to localize lesions exposed on both the visceral and the parietal pleural surfaces. Moreover, differentiation between pl0 and pl1 lesions in primary lung cancer was suggested to be useful in terms of determining therapeutic strategies including surgical procedures. However, borders of lesions were unclear on some images; thus, further innovations to improve the accuracy of the system are awaited.

Disclosure Statement

There is no conflict of interest regarding to this manuscript.

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