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Original article

Photodynamic diagnosis of visceral pleural invasion of lung cancer with a combination of 5-aminolevulinic acid and autofluorescence observation systems

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A B S T R A C T

Background: Visceral pleural invasion (PL) is a prognostic factor in lung cancer. In the lung, lymph flows along the pleura, in addition to the flow toward the pulmonary hilum just as the pulmonary arteries and veins run toward it. Even with the same tumor diameter, a PL1 or higher level of pleural invasion is indicative of a more advanced disease stage. Final diagnosis based on the PL level is made by pathological examination of excised specimens. However, if an intraoperative diagnosis can be established, proper selection of the surgical procedure can be made, and unnecessary surgeries for disseminated lesions can be avoided. We investigated optical diagnostic techniques for identifying the presence or absence of visceral pleural invasion in lung cancer by capitalizing on the phenomenon of 5-amino-levulinic acid (5-ALA) being metabolized to a photosensitizing substance or protoporphyrin IX within malignant tumors, generating red luminescence in response to excitation light.

Method: This study included 38 patients with primary lung cancer who underwent surgery. They received 5-ALA (20 mg/kg) orally 4 h before surgery and then we assessed the presence or absence of pleural invasion using an autofluorescence observation system. At visceral pleural invasion sites, we were able to confirm tumor sites visualized in red with a clear border in contrast to the green autofluorescence generated in normal tissues.

Result: Red luminescence could be confirmed in 100% of PL1-PL3 patients (14/14) and 41.6% of PL0 patients (10/24) with primary lung cancer. PL0 patients in whom visualization was possible were preoperatively diagnosed as having PL1 and many of them showed vascular channel invasion. The sensitivity, specificity, positive predictive value, and negative predictive value of this diagnostic technique were 100%, 58.0%, 63.1%, and 100%, respectively. Red fluorescence emission was observed significantly more often in pleural invasion cases.

Conclusion: Accurate intraoperative diagnosis for visceral pleural invasion in lung cancer may contribute to determining the indications for limited operations such as segmental resection. In addition, accurate local diagnosis has the possibility of being applicable to photodynamic therapy.

1. Introduction

In the lung, lymph flows along the pleura, in addition to the flow toward the pulmonary hilum just as the pulmonary arteries and veins run toward it. Visceral pleural invasion means that the disease stage is more advanced even if the tumor diameter is the same, and pleural invasion (PL) is one of the prognostic factors of disease progression in lung cancer. Since intraoperative macroscopic diagnosis and diagnostic imaging techniques such as computerized tomography (CT), magnetic resonance imaging (MRI), and positron emission computerized-tomography (PET) have limitations, more accurate diagnostic methods have been eagerly awaited. Currently, the final diagnosis is made by pathological examination of excised specimens, but if intraoperative diagnosis is possible, selection of the optimal surgical procedure becomes possible. We have been investigating a novel photodynamic diagnosis (PDD) technique that uses an autofluorescence observation system, focusing particularly on autofluorescence [1], and have been making efforts to achieve further accuracy because the visualization of lesions

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and the border between normal tissues and malignant lesions remain unclear. We thus turned our attention to a photosensitizer, i.e. 5-aminolevulinic acid (5-ALA). Once 5-ALA is ingested exogenously, it is metabolized to a heme precursor or protoporphyrin IX, which stays within malignant cells and emits red fluorescence at 630 nm [2]. This form of research is currently being carried out in the field of thoracic surgery [3] focusing on topics such as malignant pleural disease, in the field of neurosurgery with targets such as brain tumors [4], and in the field of urology for diseases such as bladder and prostate cancers [5,6]. After patients with lung cancer had ingested 5-ALA orally, we compared the diagnosis of lesions with visceral pleural invasion and the pathological diagnosis of the resected lesions to assess the usefulness of this novel method. Reports are also found in which visceral pleural invasion factors influence prognosis [7,8]. The purpose of this study is to investigate whether intraoperative judgment of pleural invasion is one factor to determine adaptation of reduction surgery for lung cancer such as segmentectomy.

2. Material and methods

From among patients with primary lung cancer who underwent surgery from July 2015 to April 2017, we enrolled 38 in whom the tumor invaded the pleura or was relatively close to the pleura on preoperative diagnostic imaging. According to the preoperative diagnosis, 13 patients were PL0, 17 were PL1, and 4 each were PL3 and PL4. Patients were administered 20 mg/kg of 5-ALA orally 4 h prior to surgery and we used a thoracoscope equipped with an autofluorescence imaging system immediately after the start of surgery to observe the pleural space through a 12 mm port hole. Observations were made in pulmonary contraction state under isolated lung ventilation. One case was stage IV with disseminated metastasis and was not resected. In all other cases, standard surgery for lobectomy and mediastinal lymph node dissection was performed. In addition of pl3 cases, the wall side pleura of the local invasion was excised in addition to standard surgery.

2.1. Autofluorescence observation system

Autofluorescence is a spontaneous emission of light generated by biological structures such as mitochondria and lysosomes when they absorb excitation light. As to the sources of autofluorescence in systemic tissues, collagen, and fibronectin, nicotinamide-adenine dinucleotide phosphate (NADPH) and flavin-adenine dinucleotide (FAD), have also been reported [9,10]. In normal tissues, green autofluorescence at about 520 nm is observed in response to a blue excitation wavelength of 400–500 nm. At the sites of cancer lesions, diminished green autofluorescence and color tone changes involving the fluorescence generated are observed due to thickening of the mucosal epithelium, a decrease in green autofluorescent substances, and increases in fluorescence absorbing substances. Imaging of this diminished fluorescence and changes in the wavelength for observation is the principle underlying the autofluorescence observation system (Fig. 1).

This study aimed to establish a diagnostic method that can contrast red sites of 5-ALA taken into malignant lesion sites in the pleural space and green autofluorescence in normal tissues, using a thoracoscope (rigid scope) equipped with an autofluorescence observation system. As to the autofluorescence observation system used in this study, we modified a conventional endoscopic color fluorescence system, PDS-2000 (Hamamatsu Photonics, Shizuoka, Japan) [11,12] and equipped it with a camera using a CCD sensor, which allowed us to observe the target with white light as well as autofluorescence through the filter (Fig. 2). A color fluorescence camera was attached to a thoracoscope using portions of the Olympus endoscopic system to achieve attachment The LED light source capable of delivering an excitation light with a peak wavelength around 405 nm was used.

2.2. 5-Ala:

5-ALA, the starting substance in the synthetic pathway of 5-porphyrin, is a natural amino acid within the body. 5-ALA is an endogenous amino acid synthesized by glycine and succinyl CoA in mitochondria and is a precursor of hemoglobin. When 5-ALA is ingested exogenously, it is rapidly metabolized to heme in normal tissues. In contrast, a fluorescent substance or protoporphyrin IX (PpIX) accumulates selectively in malignant cells because they have high porphobilinogen deaminase (PBGD) activity and low ferrochelatase (FECH) activity. As a result, red fluorescence at about 630 nm is emitted in response to an excitation light wavelength of about 405 nm (Fig. 3). In this study, we diagnosed lesions by contrasting green autofluorescence and red fluorescence emitted by 5-ALA to determine the presence or absence of visceral pleural invasion in lung cancer.

2.3. PL category

The PL category was determined by diagnostic imaging (CT diagnosis), and the pl category was then confirmed by pathological examination after surgery. Preoperative diagnosis was determined using CT photograph in consideration of the contact condition of the tumor to the pleura. PL categories is shown in Table 1, and refers to TNM Classification for Non-Small Cell Lung Cancer [13]. This study was approved by the Ethics Committee of the Asahikawa Medical College. Informed consent was obtained from each patient prior to surgery. Statistical processing was done by Chi-squared test

3. Results

There were no adverse events attributable to oral administration of 5-ALA.

1) Degree of visualization: Even when lesions were indistinguishable under white light in patients with pleural invasion, the autofluorescence camera visualized tumor sites in red with a clear border in contrast to the green autofluorescence generated in adjacent

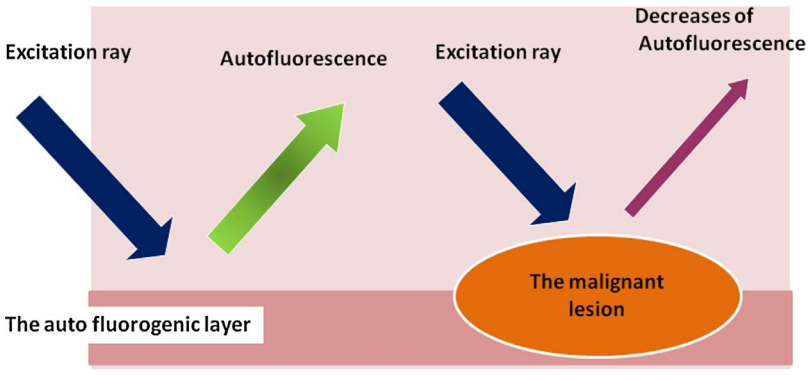

Fig. 1. The principle of autofluoresence observation.
Normal tissues: Green autofluorescence at about 520 nm was observed in response to a blue excitation light at about 400–450 nm. Lesion sites: Green autofluorescence diminished and the color tone of the generated fluorescence changed due to thickening of the mucosal epithelium, a decrease in autofluorescent substances, and an increase in fluorescence absorbing substances