Increased Microcirculation in Subepithelial Invasion of Lung Cancer

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Abstract

Background and Objective  Mucosal irregularity and hypervascularity associated with primary lung cancer in large airways are observed by bronchoscopy. The aim of this study was to evaluate microcirculation at subepithelial invasion sites of lung cancer.

Methods and Patients  Between July 2001 and June 2007, 12 patients who had subepithelial invasion sites of lung cancer in the large airways (aged 52 to 74 years, 12 males) were enrolled into this study. They were 6 patients with adenocarcinoma, 4 patients with squamous cell carcinoma, and 2 patients with small cell carcinoma. We compared 12 control subjects without endobronchial abnormality (aged 51 to 83 years, 9 males and 3 females). The patients underwent conventional bronchoscopy and subsequent high magnification bronchovideoscopy with the conventional imaging and the narrow band imaging (NBI). For evaluating microcirculation of subepithelial invasion, hemoglobin index was calculated.

Results  In high magnification view, aberrant microvessels and/or irregular mucosal thickening were observed at subepithelial invasion sites of lung cancer. Irregularly enlarged microvessels were increased and formed an aberrant microvessel network on the surface of irregular mucosa. The diameter of aberrant microvessels was significantly increased compared to normal microvessels. By switching to NBI, the aberrant microvessels were more clearly visualized. The levels of hemoglobin index were significantly higher in subepithelial invasion sites of lung cancer compared to normal mucosa.

Conclusion  In subepithelial invasion of lung cancer, aberrant microvessels are thought to be characteristic and subepithelial microcirculation may be increased.

Key words: high magnification bronchovideoscopy, microvessel diameter, hemoglobin index, narrow band imaging


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Introduction

Lung cancer causes a variety of mucosal changes when it extends to the bronchus. Angiogenesis is recognized as increased vascularity or reddish color in bronchial mucosa by bronchoscopy. It was reported that many cases of squamous dysplasia had vascular patterns of increased vessel growth and complex networks of tortuous vessels in the bronchial subepithelial layer in an early stage of lung cancer (1). Subepithelial invasion of lung cancer is often accompanied by proliferation of vessels within the neoplastic interstitial tissue, while the individual cancer cells infiltrate and destroy normal mucosal structure. However, microcirculation in subepithelial invasion sites has not been fully investigated.

Recently, high magnification bronchovideoscopy in combination with narrow band imaging (NBI) system has been reported to be useful for the detection of aberrant microvessels in dysplastic or neoplastic lesions in central lung cancer (2). NBI is a new device which improves the image quality of the superficial structures of mucosa. The technology was designed by consideration of the wavelength dis-

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crepancy of light penetration depth to tissues (3, 4). The NBI system is able to obtain fine images of vasculature or mucosal surface structures. In addition, mucosal hemoglobin content is quantitatively estimated by hemoglobin index. The hemoglobin index has been defined as endoscopic measurement of hemoglobin content in mucosa (5-7).

Herein, we observed increased aberrant microvessels on subepithelial invasion of primary lung cancer in large airways by side-viewing high magnification bronchovideoscope in combination with NBI. In order to evaluate subepithelial microcirculation, the levels of hemoglobin index were compared between a subepithelial invasion site of lung cancer and normal mucosa by using computer software.

Patients and Methods

Patients

Between July 2001 and June 2007, 12 patients with primary lung cancer (aged 52 to 74 years, 12 males) were enrolled in this study. Primary lung cancer included 6 patients with adenocarcinoma, 4 patients with squamous cell carcinoma, and 2 patients with small cell carcinoma. According to bronchoscopic findings, angiogenesis was observed in 7 cases, mucosal thickness was observed in 4 cases and both were observed in one case.

We determined subepithelial invasion sites of lung cancer from bronchoscopic findings: the pale epithelial surfaces, vagueness of groove between the mucosal folds, disappearance of the longitudinal mucosal foods, and/or blurring of the intercartilage groove. These irregularities of the mucosal surfaces were observed although normal sheen was preserved (8). In addition, we excluded epithelial invasion sites of lung cancer in bronchoscopic findings.

Next, we compared the patients with 12 control subjects without endobronchial abnormality (aged 51 to 83 years, 9 males and 3 females). The control subjects had no history of smoking and no abnormal finding in large airways in the examination of conventional bronchoscopy. None of the patients was on regular medication. We excluded patients with infectious diseases, allergic diseases, collagen diseases, and granulomatous diseases in large airways. The lesions were observed with the high magnification bronchovideoscope after observation with the conventional bronchovideoscope. All patients gave informed consent before bronchoscopic examination. The Sapporo Medical University Human Ethics committee approved this study.

High magnification bronchovideoscopy

The high magnification bronchovideoscope (XBF200HM 3, XBF-240HM5, Olympus Medical Systems Corp., Tokyo, Japan) is 6.2 mm in diameter (9). This flexible bronchovideoscope is a side-viewing type and equipped with a charge-coupled device (CCD) at its distal end. The images of bronchial mucosa are passed into the CCD through a magnifying objective lens. As with the conventional bronchovideoscope, optical information is converted to electric signals by CCD, and transmitted to the video processor, EVIS CV-240 (Olympus Medical Systems Corp.). The signals from the high magnification bronchovideoscope were re-constructed to the visual signals by a CV-240 and projected onto a 14-inch video monitor. Observation depth is 1 to 3 mm, and the images of bronchial mucosa were visualized with 55 to 110 times magnification power on a 14-inch video monitor according to the distance between the objective lens and bronchial surface. The view angle is 45 degrees.

NBI system

NBI is a new optical technology that can clearly visualize the microvascular structure on mucosal surfaces. The NBI system is based on a modification of the spectral features with an optical color separation filter which narrows the bandwidth of the spectral transmittance. The filter is placed in the optical system of the illumination. The filter cuts all wavelengths in illumination except for two narrow wavelengths of 400-430 nm (NBI-B filter) and 530-550 nm (NBI-G filter). The NBI color images are reproduced in the processor with the information from illumination of the two bands (10).

This system (XCLV-260HP, Olympus Medical Systems Corp.) is equipped with a Red/Green/Blue sequential illumination light source (400-700 nm wavelength), and a video-processor (XCV-260, Olympus Medical Systems Corp.). The light source has an optical filter for NBI. When its observation mode is NBI, the NBI filter is inserted into the optical axis of the light source. Switching between conventional imaging and NBI is achieved by pressing a button on the light source or on the control section of the scope during the procedure.

Procedures

We observed the trachea and extrapulmonary bronchi of the patients under local anesthesia, as in routine bronchoscopy. Subsequent high magnification bronchovideoscopy was performed with conventional imaging and NBI.

In brief, after muscle injection of atropine sulfate, 0.5 mg, and pentazocin, 15 mg, and nebulization of 12 mL of a 2% lidocaine solution, a conventional bronchovideoscope was orally inserted into the trachea under local anesthesia and the endobronchial lumen was examined. Next, we observed endobronchial subepithelial invasion sites of primary lung cancer under high magnification with conventional imaging and NBI. In addition, intercartilage portions of the large airways were observed in control subjects. The bronchoscopic images were digitally recorded and compared after the bronchoscopic examination.

Calculation of microvessel diameter

We defined an aberrant microvessel as a tortuous microvessel forming complex networks on a subepithelial invasion site of lung cancer. The observation depth with high magnification bronchoscope is 1-3 mm. Using known charts,
Fig 1. A 54-year-old man with adenocarcinoma, conventional view (A) and high magnification view (B: conventional imaging, C: narrow band imaging). Arrow shows observation site of high magnification bronchovideoscope. The subepithelial invasion with increased aberrant microvessels was observed in the membranous portion of the intermediate bronchus. Fine aberrant microvessels were observed more clearly in narrow band imaging as compared to conventional imaging (white dot circle). In addition, a region of interest was determined as the rectangle area and the hemoglobin index was calculated. Schematic color display of the hemoglobin index subjectively identified distinct redness in subepithelial invasion site by using computer software (D).

magnification on a 14-inch TV monitor was calculated at 55 to 110 times. We calculated the diameter of aberrant microvessels and that of normal microvessels by using Adobe Photoshop 6.0 (Adobe Systems Incorporated, San Jose, CA, USA). When vessel diameter is expressed as pixel distance \((D)\) between two points, \((X_1, Y_1)\) and \((X_2, Y_2)\), on Adobe Photoshop 6.0, actual vessel size \((L)\) is expressed as follows:

\[
L = K \times D / M
\]

\(K\): 1.28×10^7 \ \mu\text{m/pixel} \ \text{(conversion factor)}

\(D\): \(\sqrt{(X_1-X_2)^2 + (Y_1-Y_2)^2}\)

\(M\): magnification

\(K\) was calculated from the number of pixels on the XY coordinates measured using actual measurement values (\(\mu\text{m}\)) on the TV monitor and Adobe Photoshop 6.0. As the actual distance between the objective lens of high magnification bronchovideoscope and bronchial mucosa was not possible to measure, we postulated that \(M\) was 110. We selected high magnification images which clearly showed subepithelial microvessels and approached the targeted bronchial mucosa as close as possible.

**Measurement of hemoglobin index**

The levels of hemoglobin index of aberrant mucosa of subepithelial invasion sites of primary lung cancer in high magnification images were compared with that of normal bronchial mucosa of control subjects. A region of interest (ROI) was determined from original high magnification images on a computer display as the rectangular area(Fig. 1D). Hemoglobin index of each image was calculated by using the appropriate computer software (SolemioENDO ProStudy, Olympus Medical Systems Corp.). In addition, in the control subjects the intercartilage portion of the trachea was culcu-
Vessel diameter (μm)  

- Normal microvessel  
- Aberrant microvessel  

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p<0.0002

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<th>Hemoglobin Index</th>
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p<0.0001

Figure 2. Diameters of aberrant and normal microvessels were calculated. The microvessel diameter was more significantly increased in the subepithelial invasion sites (57.0 μm; IQR: 39.5-85.4) than in the normal bronchial mucosa (34.4 μm; IQR: 27.7-45.6; p<0.0002). Data are expressed as median and inter-quartile range (IQR).

Statistical analysis

Results were expressed as median and inter-quartile range (IQR). Differences in the diameter of subepithelial microvessel and in the level of the hemoglobin index were compared using the Mann-Whitney U test. Differences were considered statistically significant when the p value was less than 0.05.

Results

Observation of subepithelial invasion of primary lung cancer with high magnification

First, we observed subepithelial invasion sites of endobronchial tumor of patients with primary lung cancer by using a high magnification bronchovideoscope with conventional imaging. Irregular mucosa and hypervascularity were observed at subepithelial invasion sites by conventional bronchovideoscope (Fig. 1A). In high magnification view, increased aberrant microvessels were observed on the surface of irregular mucosa (Fig. 1B). These microvessels were found to meander and were irregularly enlarged.

By changing from conventional imaging to NBI, these aberrant microvessels were more clearly and sharply visualized in the NBI as compared to the conventional imaging (Fig. 1C). Fine microvessels were clearly visualized as brown vessels and thick microvessels were visualized as light-green vessels on the NBI images. Brown vessels were visualized in the NBI-B filter image, and light-green vessels were visualized in the NBI-G filter image.

Measurement of diameter of aberrant microvessels

First, 10 magnification images from the 5 patients with primary lung cancer (2 adenocarcinomas, 2 small cell carcinomas and one squamous cell carcinoma) and 10 images from the 5 control subjects were selected. Next, 30 aberrant microvessels from the subepithelial invasion sites of lung cancer and 30 normal microvessels from the normal tracheal mucosa were selected. The diameter of these microvessels was calculated. The microvessel diameter was more significantly increased in the subepithelial invasion site (57.0 μm; IQR: 39.5 -85.4) than in the normal bronchial mucosa (34.4 μm; IQR: 27.7- 45.6; p<0.0002, Fig. 2). In addition, there was no significant difference in aberrant microvessel diameter between histological types of lung cancer.

Hemoglobin index of subepithelial invasion of lung cancer

Thirty-nine high magnification images from the 12 patients with primary lung cancer and 38 images (trachea; 30 images, main bronchi; 8 images) from the 12 control subjects were analyzed. The levels of hemoglobin index of high magnification images of subepithelial invasion site of lung cancer and normal subjects were calculated. The level of hemoglobin index was significantly higher in the subepithelial invasion of primary lung cancer (67.9, IQR; 58.3-77.9) than in the normal subjects (49.1, IQR; 44.8-52.9; p<0.0001). Data were expressed as median and IQR.

Discussion

Angiogenesis is necessary for malignant neoplasm, because the hyperproliferated state requires an adequate blood supply. In the present study, we observed subepithelial invasion of primary lung cancer by using a high magnification bronchovideoscope. Proliferated aberrant microvessels were identified and the microvessels were irregularly enlarged and were found to meander on irregular mucosa. The aberrant microvessels were connected to each other, and thought to connect to the surrounding normal subepithelial microvessels. The characteristics of these aberrant microvessels were thought to be unequal diameter, tortuous shape and aberrant network. In addition, these aberrant microvessel diameters were significantly increased as compared to normal microvessels. Aberrant microvessels at subepithelial invasion sites were thought to provide nutrient blood flow to lung cancer and play an important role to maintain tumor growth.

A combination of high magnification bronchovideoscopy with NBI yielded clear images of the microvasculature network with an increased contrast. In particular, fine aberrant microvessels were depicted as brown microvessels by the NBI-B filter. Judging from the spectral feature, it was speculated that the brown vessels were located beneath the mucosal surface. An approach to visualize angiogenesis or aberrant microvessels in the subepithelial cancerous lesion can be a new diagnostic method. The clinical usefulness of NBI combined with magnifying endoscopy has been reported in the larynx (11), bronchus (2, 9), and gastrointestinal tract (12-15). These reports evaluated the ability to detect early cancer in the basis of the vascular irregularities.

The levels of hemoglobin index were significantly increased in subepithelial invasion sites of lung cancer than in normal mucosa. This result was thought to mean an increase of microcirculation in the subepithelial invasion. Conventionally, the endoscopic color of bronchial lesion has been described as reddened, the same, or discolored when compared with the color of the surrounding normal mucosa. Such descriptions have been subjective and it was impossible to measure color quantitatively. Hemoglobin is the predominant pigment in the mucosa. Hemoglobin index can quantitatively express the levels of vascularity in the subepithelial invasion of lung cancer. The measurement of hemoglobin content using the hemoglobin index might facilitate evaluation of vascularity or microcirculation in subepithelial invasion.

Angiogenesis is critical to the transition of premalignant lesion to the malignant phenotype. Observations of subepithelial microvessels may provide important information on the manner in which neovascularization progresses. The limitation of our study was that we were not able to focus on the deeper layer of the epithelium. The subepithelial microvessel network might correlate with the depth of tumor invasion.

The authors state that they have no Conflict of Interest (COI).

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References