Studies on autofluorescence of gastric cancer and imaging of pancreaticobiliary diseases

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Chapter 3

AUTOFLUORESCENCE IMAGING ANALYSIS
OF GASTRIC CANCER

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ABSTRACT

Aims: To investigate the characteristics of gastric cancer in autofluorescence images.

Methods: A double-channel laser scanning confocal microscope with an argon ion laser (excitation wavelength 488nm) and helium-neon laser (excitation wavelength 543nm) were used to detect the autofluorescence from 16 gastric cancer tissue specimens and corresponding normal gastric tissue.

Results: Autofluorescence from normal gastric tissue produced a green image. The intensity of red color increased obviously in all gastric cancer tissue (100%) after illuminating and the tissues produced a reddish-brown-colored image.

Conclusions: A reddish-brown image is characteristics of autofluorescence in gastric cancer detected by an argon ion laser and helium-neon laser with a double-channel laser scanning confocal microscope. Autofluorescence imaging analysis is useful in the diagnosis of gastric cancer.

Key Words: Gastric cancer  Autofluorescence
Laser scanning confocal microscope
INTRODUCTION

Laser scanning confocal microscope (LSCM) uses a fluorescence microscope with a laser scanning device together with computer image-processing techniques to obtain fluorescence images of subtle tissue structures \(^{[1,2]}\). The aim of the present study was to investigate the characteristics of gastric cancer by using LSCM to detect autofluorescence from surgical specimens of gastric cancer tissues and corresponding normal gastric tissues.

MATERIALS AND METHODS

Sixteen histologically diagnosed surgical specimens of adenocarcinomatous gastric cancer tissue were examined using LSCM autofluorescence imaging. Of the specimens, seven were poorly differentiated, six were moderately differentiated, and three were highly differentiated.

Samples of cancerous and normal tissue were obtained from fresh surgical specimens. Normal controls were taken from resection specimens at least 5 cm away from cancerous lesions. All specimens of gastric cancer and corresponding normal tissue were approximately 0.5 cm x 0.5 cm in size with three layers: mucosa, submucosa and muscularis propria. The specimens were immediately frozen without any pretreatment. Cancerous and adjacent normal gastric tissue sections were stained with hematoxylin and eosin (H&E) and submitted for routine histological examination.

Double-channel LSCM (Zeiss 510) was used to detect the green and red autofluorescence from gastric cancer tissue and all layers of the normal gastric tissue, comprising mucosa, submucosa and muscularis. The LSCM scanning area was approximately 0.22 \(\mu m^2\) with an imaging matrix of 512\times512. Two excitation lights were selected: one was a 15-mW argon ion laser (excitation wavelength = 488 nm) with green autofluorescence at wavelengths between 505 and 530 nm, the other was a 0.5-mW helium-neon laser (excitation wavelength = 543 nm) with red autofluorescence at wavelengths greater than 580 nm. For each specimen, green, red and the integrative images were stored.

In addition, the autofluorescence intensity was also measured using software designed for the purpose (Zeiss version 2.10). The green/red autofluorescence intensity ratio was calculated in the cancerous and normal controls. The two groups were compared using a one-tailed unpaired Student's \(t\)-test.
RESULTS

Autofluorescence images of gastric cancer tissue and normal tissue are shown in Fig 1 and Fig 2. Green autofluorescence was seen in all three layers of the normal gastric tissues; in the integrative image of the green and red autofluorescence, the green color was evenly distributed.

The green/red autofluorescence intensity ratios in normal gastric mucosa, submucosa and muscularis were 3.20, 3.98 and 4.12, respectively. The intensity of red autofluorescence increased in all gastric cancerous tissues compared with the normal tissue, and the cancerous lesions were seen as a reddish-brown-colored area (Fig 3). There were no false-positive or false-negative results.

The green/red autofluorescence intensity ratio in gastric cancer tissues was 1.29, which was significantly lower from that of normal gastric tissue ($P<0.01$; Fig 4).

![Figure 1](image)

**Figure 1.** A B C

Autofluorescence image of normal gastric mucosa. **(A)** red autofluorescence image produced by helium-neon laser; **(B)** green autofluorescence image produced by argon ion laser; **(C)** integrative image of green and red autofluorescences produced by both helium-neon laser and argon ion laser simultaneously (evenly distributed green color).
Auto-fluorescence image of gastric cancer tissue. **(A)** red auto-fluorescence image produced by helium-neon laser; **(B)** green auto-fluorescence image produced by argon ion laser; **(C)** integrative image of green and red auto-fluorescence produced by both helium-neon laser and argon ion laser simultaneously. The cancerous lesion is shown as reddish-brown-colored area.

**Figure 3.** Histology shown as poorly differentiated adenocarcinoma of stomach (hematoxylin and eosin stain, ×330). The auto-fluorescence image of this specimen is shown in Fig 2.
Figure 4. Comparison of the green/red autofluorescence intensity ratio of gastric cancerous lesions and three layers of normal gastric tissue ($P<0.01$).

DISCUSSION

The autofluorescence technique is a noninvasive, safe, simple and fast procedure that can be used in the diagnosis of gastric cancer. With the advantages of real-time detection of lesions and its ability to serve as a guide for taking biopsy specimens, it plays an important role in diagnosing precancerous lesions and the early stages of gastrointestinal cancer efficiently.$^{[3-7]}$

Recently, endoscopic autofluorescence imaging systems have been used to diagnose gastrointestinal cancer, and preliminary clinical usage has indicated that this newly developed system is valuable in detecting dysplasia and early gastrointestinal cancers. The laser-induced fluorescence endoscope (gastrointestinal) (LIFE-GI) consists of a mercury light source with blue light pass filter, a camera equipped with two high-sensitivity image sensors for the detection of green and red autofluorescence, and an imaging processor for displaying autofluorescence image. Yano et al. used the LIFE-GI system to test 30 patients with gastric cancer or suspicious gastric cancer$^{[8]}$. In 23 of 25 early gastric cancers, the lesions could be observed as a dark reddish-colored area; histological examination revealed that the tumors corresponded well with this dark-reddish-colored area$^{[8]}$. Van Ierland-van Leeuwen and Tytgat and his group$^{[9,10]}$ used LIFE-GI system to examine 38 patients with regular endoscopy, including 23 colonoscopies and 15 gastroscopies; they found that autofluorescence of dysplasia and carcinoma in situ was seen as a reddish-brown to red color$^{[9,10]}$. Autofluorescence images and histology were compared and the diagnostic sensitivity of this method was found to be 100%; no false-negative results were obtained$^{[9,10]}$. 
They also diagnosed 2 carcinomas in Barrett's segment, which were not clearly identified during regular endoscopy. In addition, the LIFE-GI system has been used in the detection of small malignant lesions, such as gastric cancer in the remnant stomach\textsuperscript{[11,12]}.

In the present study, we detected the autofluorescence in 16 surgical specimens of gastric cancer using a double-channel laser scanning confocal microscope to investigate the characteristics of autofluorescence images in gastric cancer. We found that 100\% of the autofluorescence in gastric cancer tissue appeared as a reddish-brown image, with no false-negative results. The reddish-brow color of the autofluorescence image of gastric cancer tissue was due to an increase in the intensity of red autofluorescence and a decrease in the ratio of green/red autofluorescence intensity compared with the autofluorescence images of normal control tissue. Autofluorescence imaging analysis could be useful in the diagnosis of gastric cancer.
REFERENCES
