Establishing diagnostic features for identifying the mucosa and submucosa of normal and cancerous gastric tissues by multiphoton microscopy

Jianxin Chen, PhD,* Shuangmu Zhuo, PhD,* Gang Chen, MD,* Jun Yan, MD, Hongquan Yang, BSc, Nenrong Liu, MSc, Liqin Zheng, MSc, Xingshan Jiang, PhD, Shusen Xie, BSc

Fuzhou, China

Background: Establishing diagnostic features is essential and significant for developing multiphoton endoscopy to make an early diagnosis of gastric cancer at the cellular level. Until now, these diagnostic features have not been clearly described and understood.

Design: Study of diagnostic features based on multiphoton microscopy (MPM).

Objective: Establishing diagnostic features to identify the mucosa and submucosa of human normal and cancerous gastric tissues by investigating their multiphoton microscopic images.

Setting: Fujian Normal University and Fujian Provincial Tumor Hospital.

Patients: Ten pairs of normal and cancerous specimens were obtained from 10 patients (ages 51-68 years) undergoing radical gastrectomy.

Interventions: MPM was performed on specimens.

Main Outcome Measurements: Establishment of diagnostic features.

Results: MPM has the ability to exhibit not only the mucosal and submucosal microstructures of normal and cancerous gastric tissues but also the distribution and content of abnormal cells in these 2 layers. More importantly, it can provide the diagnostic features to qualitatively and quantitatively differentiate between normal and cancerous gastric tissues.

Limitations: The selection bias and preparation of specimen.

Conclusions: These findings provide the groundwork for further establishing diagnostic criteria. (Gastrointest Endosc 2011;73:802-7.)

Endoscopically determined diagnosis in real time in vivo at the cellular level is the clinical key to increasing the survival rate of early gastric cancer. Confocal laser endomicroscopy can allow high-resolution in vivo histology

Abbreviations: GGO, gastric gland orientation; MPM, multiphoton microscopy; SHG, second harmonic generation; TPEF, 2-photon excited fluorescence.

DISCLOSURE: *Drs Chen, Zhuo, and Jiang were supported by the National Natural Science Foundation of China (grants 30970783, 60908043), the Program for New Century Excellent Talents in University (grant NCET-07-0191), the Natural Science Funds for Distinguished Young Scholar in Fujian Province (grant 2009J06031). Dr Yan was supported by the Natural Science Foundation of Fujian Province (grant 2010J01136). All authors disclosed no financial relationships relevant to this publication.*

*Drs J. Chen, S. Zhuo, and G. Chen contributed equally to this article.

0016-5107/$36.00
doi:10.1016/j.gie.2010.12.016

Received September 14, 2010. Accepted December 10, 2010.

Current affiliations: Institute of Laser and Optoelectronics Technology (J.C., S.Z., H.Y., N.L., L.Z., X.J., S.X.), Fujian Provincial Key Laboratory for Photonics Technology, Key Laboratory of OptoElectronic Science and Technology for Medicine of Ministry of Education, Fujian Normal University, Fuzhou, China, Department of Pathology (G.C.), Fujian Provincial Tumor Hospital, Fuzhou, China, Department of Surgery (J.Y.), Fujian Provincial Tumor Hospital, Fuzhou, China.

Reprint requests: Jianxin Chen, PhD, Fujian Normal University, 8 Shangsan Road, Fuzhou, China 350007.

If you would like to chat with an author of this article, you may contact Dr Chen at chenjianxin@fjnu.edu.cn, or Dr Yan at ynjun@yahoo.com.

assessment.[1,2] Moreover, confocal reflectance microscopy shows the potential for diagnosing cancer without the use of contrast agents.[3] However, the emergence of multiphoton microscopy (MPM) based on intrinsic 2-photon excited fluorescence (TPEF) and second harmonic generation (SHG) has attracted more attention. TPEF is a nonlinear process in which the fluorophore absorbs 2 photons with lower energy simultaneously and emits a single photon of fluorescence with higher energy. SHG is a coherent scattering process in which 2 photons with lower energy are combined to create a single photon of exactly twice the lower energy.[4,5] These elements of MPM offer significant advantages over confocal imaging techniques for imaging in thick tissue and live animals, such as greater imaging penetration depth and reduced out-of-focus photobleaching and phototoxicity, which permit more long-term fluorescence observation.[6-8] Recently, it has been applied to imaging the gastric mucosa[9,10] and was developed into a multiphoton endoscope for neurobiology research.[11,12] In addition, the miniaturized multiphoton tomography DermaInspect (JenLab GmbH, Jena, Germany) and multiphoton probe allows the clinical use of multiphoton endoscope for diagnosing cancer.[9,13,14] Thus, establishing diagnostic features is essential and significant for developing multiphoton endoscopy to allow early diagnosis of gastric cancer. Until now, these diagnostic features have not been clearly described and understood. In this report, we attempted to establish diagnostic features to distinguish between normal and cancerous gastric tissues.

Take-home Message

- Establishing diagnostic features is essential and significant for developing multiphoton endoscopy to make an early diagnosis of gastric cancer at the cellular level. The authors' results demonstrated that multiphoton microscopy has the ability to provide the diagnostic features to qualitatively and quantitatively differentiate between normal and cancerous gastric tissues.

MATERIALS AND METHODS

Sample preparations

Ten pairs of normal and cancerous specimens with mucosal and submucosal structures 1 to 1.5 cm wide and 0.2 cm thick were obtained from 10 patients (ages 51-68 years) undergoing radical gastrectomy at the Fujian Provincial Tumor Hospital. Written informed consent was obtained from each patient. The normal gastric tissue was 6 cm away from the cancer margin. Each gastric specimen was divided into 2 parts: 1 part of each specimen was cut into 5-μm transverse tissue slices for MPM imaging and 1 part was stained with hematoxylin and eosin for histological images, showing that 10 pairs of gastric specimens were 10 normal tissue and 10 were cancerous tissue, respectively. During our investigations, we usually confirmed the observed results from the MPM images by comparing with the histological images of paired sections.

The MPM microscopic imaging system

The multiphoton microscope used in this study was described previously.[15] In short, the TPEF/SHG images were acquired by using an LSM 510 META system (Zeiss, Jena, Germany) coupled with a Ti:sapphire laser (Mira 900-F; Coherent Inc, Santa Clara, Calif). An oil immersion objective (Plan-Apochromat 63×, NA 1.4; Zeiss) was used for focusing the excitation beam on the samples and collecting the backscattered TPEF/SHG signals. Each channel of the META detector covers a spectral width of approximately 340 nm (range 377-716 nm) and the detected wavelength range depends on the collected signals. In this study, to simultaneously obtain high-contrast TPEF and SHG images, the 800-nm excitation light was chosen. Two channels were selected to image collagen and fluorescence components, respectively. One channel corresponding to the wavelength range of 387 to 409 nm showed the microstructure of collagen, whereas another channel covered a range from 430 to 708 nm to collect TPEF signals. The TPEF images of intrinsic components were revealed in the first column (green color coded) and the SHG images of collagen were shown in the second column (red color coded), whereas the overlay image showing their high-contrast images were displayed in the last column in Figures 1 and 2. All images had a 12-bit pixel depth. The images were obtained at 2.56 μs per pixel.

Quantification of morphological features

To quantitatively describe the differences in morphological features between normal and cancerous gastric tissue, the nuclear area was defined as the area of nuclear boundary. The collagen area was defined as the ratio of the SHG pixels over the whole pixels in each image to show the variation of collagen. Gastric gland orientation (GGO) was defined as the angle difference between gastric glands. Specifically, the inclination angle between each gastric gland relative to vertical axis was defined as each gastric gland direction angle (<90 degrees); the angle difference was the difference between 2 adjacent direction angles. In this study, each quantitative analysis was performed on all the samples by 2 experienced individuals in identification of MPM images. For each sample, 3 random positions were selected.

RESULTS

MPM images of normal gastric mucosa and submucosa

Figure 1 shows the representative TPEF/SHG images of normal gastric mucosa and submucosa. Three rows from