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Screening of gastric carcinoma cells in the human malignant gastric mucosa by confocal Raman microspectroscopy

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Abstract

Confocal Raman microspectroscopy was used to characterize gastric carcinoma cell in both cultured cells and human gastric mucosa tissues. Based on the spectra of single cultured cell, gastric carcinoma cells were screened out in the malignant gastric mucosa successfully and the positive ratio is about 58.06%. The high SNR (signal-to-noise) spectra from human gastric mucosa tissues and cells were obtained by this technique without any sample preparation and the time of detection required less than 3 min. Comparing spectra of malignant gastric mucosa tissues with those of counterpart normal ones, there were obvious spectral changes, including intensity decrease at ~1587 cm^{-1} and alteration of peak shape at ~1660 cm^{-1} with malignancy. Additionally, spectral features of single cell also differed from those of stomach tissues at ~1525 and ~1156 cm^{-1} where these two bands were assigned to carotenoids. These results demonstrate the possibility of a rapid clinical diagnosis of gastric carcinoma with Raman microspectroscopy in combination with a remote optical probe.

Keywords: Confocal Raman microspectroscopy; Gastric carcinoma; SGC7901; Tissues; Single cell; Diagnosis

1. Introduction

Gastric adenocarcinoma is one of the leading causes of cancer death in the world, accounting for an estimated 10% of all malignancies worldwide [1]. The improvement on survival rate of this cancer has been achieved primarily through early detection and treatment. Therefore, finding an effective tool to detect cancer in its early stages is of great importance.

Medical diagnostic techniques useful for gastric carcinoma screening include barium meal, gastric endoscopy, exfoliative cytology examination, immunodiagnosis and histopathological examination. The histopathological examination is recognized as a gold standard method providing valuable clinical information but requiring time-consuming tissue processing, generally 2–3 days. Furthermore, the use of fluorescence spectroscopy with endoscopy to detect early carcinomas and to discriminate between normal tissue and neoplastic lesions has attracted extensive attention although resting on laboratory research level [2,3]. However, detecting the difference in autofluorescence as an endoscopic image has been very difficult up to the present because of its faintness and insufficient information [4].

Raman microspectroscopy is a non-destructive technique that provides information about the molecular structure of the investigated sample. The positions and relative intensities of the various spectral bands can be used to probe primary, secondary, tertiary, and quaternary structures of large biological molecules. The fingerprint spectral region, from approximately 600 to 1800 cm^{-1}, contains a series of sharp bands that can be used to characterize a particular molecule and in some cases to identify the composition of complex tissues samples. The molecular composition and/or structure of nucleic acids, some types of proteins and lipids differ between normal and tumor tissues, and therefore, Raman spectroscopy has been considered promising for the diagnosis of cancer [5]. Due to continuing

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advances in instrumentation, Raman microspectroscopy combines high spatial resolution inside single cell with detailed spectral information [6]. Hawi firstly used Raman microspectroscopy to characterize normal and malignant hepatocytes in both cultured and human liver tissues [7]. Wu and co-workers utilized FT-Raman spectroscopy to distinguish normal to malignant stomach tissue samples [8,9]. The method they acquired average spectra from gastric mucosa samples sized 0.5 cm × 0.5 cm × 0.5 cm is unsuitable for gastric cancer cells screening, furthermore not applicable for clinical diagnosis. The present work investigated the ability of the confocal Raman microspectroscopy to characterize gastric carcinoma cell in both cultured cells and human gastric mucosa tissues. Based on spectra of both cultured cell line and gastric mucosa, gastric carcinoma cells were attempted to be screened out from malignant gastric mucosa.

2. Experimental

2.1. Preparation of cell samples

Gastric carcinoma cell line SGC7901 [10] was obtained from CCTCC (China Center for Type Culture Collection). Cell suspensions were washed twice with PBS (pH 7.4) and resuspended in PBS (pH 7.4), and the suspension was diluted to an optical density of ∼3.0. This cell suspension were dropped on carriers of gold sheet for Raman spectroscopy and discarded after use [11]. Fig. 1 displays the micro-image separated SGC7901 cells dropped on carrier of normal glass.

2.2. Preparation of gastric mucosa tissue samples

Ethical approval: This protocol was approved by the relevant Local Research Ethics Committees (PR China).

Excision specimens from 15 histologically proven adenocarcinoma of stomach were obtained from 15 individuals from Department of Oncology of Zhongnan Hospital, Wuhan University, after informed consent. A biopsy was taken from each sample, snap-frozen in liquid nitrogen and stored at −80 °C until use. Cryosections (25 μm thickness) were obtained from the biopsy specimens and placed on gold sheet for Raman spectroscopy. During the measurement, the gastric mucosa tissue section was thawed to reach room temperature in air. Histopathological analysis has been performed on haematoxylin and eosin stained sections by a single GI-registry pathologist.

2.3. Spectroscopic instrumentation

A Renishaw Raman microspectrometer (Renishaw Raman system RM1000) has been optimised for maximum throughput, detection sensitivity and fluorescence suppression. The argon ion laser provided a 20 mW incident light at 514.5 nm. After attenuation through prisms and filters, the power of laser exposed on samples was only about 4 mW, which almost impossible lead to degradation of the samples. Spectra were measured from tissue and cell specimens with a ×20 short-working-distance objective (NA 1.20), and the signals were integrated for 30–120 s and measured over a spectral range of 600–1800 cm^{-1} with respect to the excitation frequency. The system includes a stigmatic spectrometer with two motorized gratings, of which the 1800 grooves/mm grating was used to provide a spectral resolution of the Raman scatter of around 4 cm^{-1}. Raman scattering was detected by using an air cooled 578 × 385 pixels CCD camera. Peak frequencies and rapid checking of instrumental performance were calibrated with the silicon phonon line at 520 cm^{-1}. Spectral data were visualized on a computer and subtracted baseline, exported TXT data by the

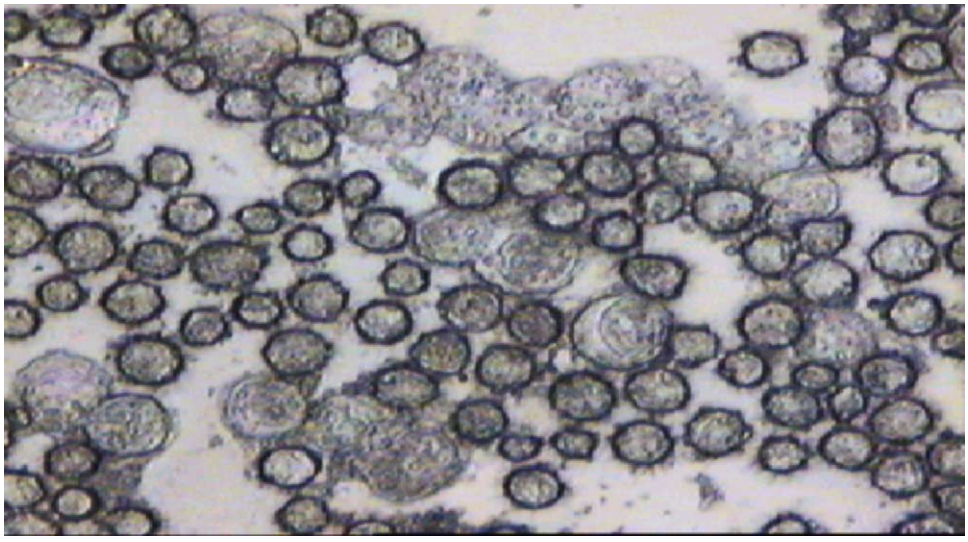

Fig. 1. The micro video image of separated SGC7901 cells on normal glass slide (×200).