Raman Spectroscopy of Ghost Cells in Calcifying Cystic Odontogenic Tumor

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Abstract: Raman spectroscopy is based on Raman scattering of light form molecules, and Raman spectra provide highly useful information about molecular composition and its circumstances. The micro FT-Raman spectra both of ghost cells in calcifying cystic odontogenic tumor and keratinocytes of the gingiva showed a broad beak centered around 850 cm⁻¹.

Key words: Raman spectroscopy, Micro FT-Raman, Ghost cell, Calcifying cystic odontogenic tumor, Odontogenic tumor

Introduction

Raman spectroscopy is one of the vibrational spectroscopic techniques and Raman spectrum contains a series of the specific and characteristic peaks and bands that are assigned to a corresponding molecular structure and biochemical composition within the tissue¹⁻³). Raman spectroscopy has been applied as a diagnostic tool for the detection of cancers, due to its sensitivity to the changes in molecular composition and conformation that occurs in malignant tissue⁴). However, there have been only a few studies using Raman analysis on oral lesions⁵,⁶).

The purpose of the present study was to clarify the Raman spectrum of ghost cells in calcifying cystic odontogenic tumor (CCOT) and compare the data to that of keratinocytes of the gingiva.

Materials and Methods

The present study used ghost cells in three cases of CCOT. Consideration was given to patient privacy, diagnosis, and the management and prognosis of the lesions (Recognition number of the ethics committee: EC 04-003). As a control, keratinocytes of the gingiva were used. Their histological slides stained with hematoxylin and eosin (HE) and covered by cover-glass were used for the following analysis. The slide without a specimen mounted by a cover-glass was also analyzed as a background.

Micro fourier transform (FT)-Raman analysis was carried out using a RXN system, Kaiser Optical System Inc., USA, equipped with an Olympus BX51 TRF microscope. Raman spectra were acquired with a Kaiser RXN1 FT-Raman spectrometer. The green laser source was a Newport invictus operated at 532 nm. An iDUS, Andor Technology, thermoelectric cooled CCD was used for detection. Rayleigh line rejection was accomplished with a HoloPlex transmissive grading, Kaiser and spectra for this work were acquired over the range of 100-4400 cm⁻¹ Raman shift. All Raman spectra were acquired at 2.5 cm⁻¹ resolution. Data acquisition was carried under the conditions of the accumulation time: 500 msec. and the accumulation number: 20 times.

Results

Raman spectra were successfully acquired from all samples stained with HE and covered by cover-glass. The spectrum of background was omitted from the spectra of specimen.

Figure 1 showed the back ground of cover glass and there were Raman shifts at 300, 500 and 1500 cm⁻¹.

Figure 2 revealed the micro FT-Raman spectra of ghost cells in CCOT and there was a broad beak with an appreciable peak at 845 cm⁻¹.

Figure 3 showed the micro FT-Raman spectra of keratinocytes of the gingiva and a broad beak with intense band at 854 cm⁻¹ was recognized.
Discussion

In recent years there has been much interest in the use of optical diagnosis in cancer detection 7). Therefore, Raman spectroscopy is used for various researches1-9), suggesting Raman spectroscopic analysis is a powerful and useful tool. There are some advantages to using Raman spectroscopy for analysis of water-rich and multi-component samples over other analytical techniques, such as infrared (IF) and ultraviolet-visible (UV-vis) abortion spectroscopies10). Furthermore, Raman spectroscopy can use the histological slides stained with hematoxylin and eosin and mounted by a cover glass2,3).

Calcifying cystic odontogenic tumor, which was formerly called calcifying odontogenic cyst, was histologically characterized by cystic proliferation of odontogenic epithelium including ghost cells. It was revealed that ghost cells were filled with many bundles of tonofilaments, suggesting they appear as a result of keratinization11). Therefore, the present study used keratinocytes as a control.

It is generally accepted that Raman has some differences as compared with FT-IR of similar spectroscopic analysis method. Raman has the following advantages: Raman can analyze small sample and the sliced specimen with cover glass, and do complementary analysis with FT-IR. Both of Raman and FT-IR can be analyzed in underwater. Raman has the following disadvantages: Since signals of Raman are generally weak, it tends to be covered with a background (spontaneous light). Raman spectroscopy instrument is more expensive than FT-IR spectroscopy.

The present study demonstrated the micro FT-Raman ability for obtaining the spectra of ghost cells in CCOT and keratinocytes of the gingiva. Both of them showed almost the same FT-Raman spectra with a broad peak having an appreciable peak at about 850 cm⁻¹. Thus, ghost cells and keratinocytes showed similar Raman spectra, suggesting both of ghost cells of CCOT and keratinocytes have similar structure as observed in TEM study10). We expect further researches can be carried out using Raman analysis method for CCOT, odontogenic tumor, oral lesions and the other disease.

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