A Study of Oral Epidermoid Carcinoma Cell Line Using Synchrotron-Based Infrared Microspectroscopy

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Abstract

Synchrotron-based infrared microspectroscopy employs a unique approach to spectral diagnosis of tissue pathology based on the characteristic molecular vibration spectra of the cell.

Materials and Methods

OECM-1 is an oral epidermoid carcinoma cell line and cultured in RPMI, 10% fetal bovine serum (FBS) and 1% Antibiotic-Antimycotic (AA) at 37℃ in a humidified atmosphere of 5% CO_2 in air. For investigating the metastasis ability of malignant tumor cells for invading the surrounding extracelluar matrix (ECM) and basement membranes, the membrane invasion culture system (MICS) was employed for simulating the environment for cancer cells invading. The invasion assay is containing polyethyleneterephthalate (PET) filters with pore size in diameter of 8 m. The matrix-coated filter was placed coated side up on the lower plate followed by carefully attaching the upper plate. Both upper and lower wells of the chamber were filled with culture medium. OECM-1 cell suspensions were seeded into the upper wells. Following 5 days incubation in a humidified incubator at 37°C with 5% CO_2, cells that had invaded through the basement membrane were collected through the side port by replacing the medium in the lower chamber with 0.25% trypsin for 5 minutes at 37°C. The cells recovered from the bottom of the filter were then loaded onto dish to fix on low-e slides used 4% paraformaldehyde for 10 －15 minutes in ice bath at 4°C then immersed in PBS for 10 min twice. These cells were then briefly washed in distilled water for removing the residual formalin and slat from the surface of the cells. OECM-1 cells were dried under ambient conditions and stored in dry box ready for spectral analysis. The samples of cell were measured by conventional SR-FTIR microspectroscopy using the aperture of 10×10 μm^2 with step size of 2 μm. Spectra were coadded for 80 scans.

Results and Discussion

Spectra were collected, baseline corrected and interpolated in the spectral region 3500-990 cm^{-1}. Representative spectra from four different location N1, N2, P1, and P2 within two single cells were shown as in the Fig. 1. The analysis of the four spectra allows the following considerations: (i) the band of 3000-2800 cm^{-1} was assign to C-H stretchingof both lipid and protein, (ii) slightly broadening for Amide I and amide II bands of both locations N1 and N2 was observed relative to locations of P1 and P2.The broadening of the Amide I band shows a change in protein conformation. The broadening in the 1636–1620 cm^{-1} region would indicate increases and/or changes in types of β-sheet structures; the broadening in the 1700–1652 cm^{-1} region would indicate some increase in random coil, α-helix and β-turn structures. However, the relative broadening of the 1563–1550 cm^{-1} region relative to the 1557–1544 cm^{-1} region of the Amide II band would suggest more β-sheet.

Chemical images, based on characteristic infrared spectra, were constructed by utilizing peak area of characteristic band of lipid at (a) 1393 cm^{-1} (lipid dominant) , (b) 1421 cm^{-1} (protein dominant), (c) 1446 cm^{-1}(protein dominant), (d) 1473 cm^{-1} (lipid dominant), (e) 2862 cm^{-1}(lipid dominant), (f) 2877 cm^{-1}(protein dominant), (g) 2925 cm^{-1}(lipid dominant), and (h) 2958 cm^{-1}(protein dominant). And for protein was at (i) 1532 cm^{-1}, (j) 1643 cm^{-1} (k) 3063 cm^{-1}, (l) 3286 cm^{-1} of OECM-1 cell and chemical images were shown in Fig. 2.

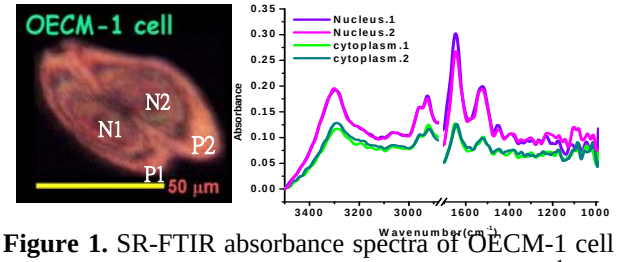

Figure 1. SR-FTIR absorbance spectra of OECM-1 cell for the spectra region between 990 and 3500 cm^{-1} and infrared image.

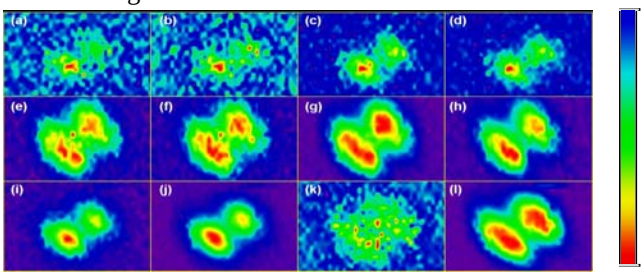

Figure 2. Chemical images were constructed by utilizing peak area of lipid at (a) 1393 cm^{-1}, (b) 1421 cm^{-1}, (c) 1446 cm^{-1}, (d) 1473 cm^{-1}, (e) 2862 cm^{-1}, (f) 2877 cm^{-1}, (g) 2925 cm^{-1}, (h) 2958 cm^{-1}. And for protein was at (i) 1532 cm^{-1}, (j) 1643 cm^{-1} (k) 3063 cm^{-1}, (l) 3286 cm^{-1} of OECM-1 cell.

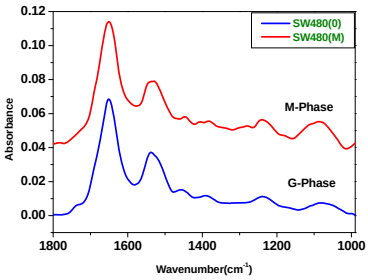

Figure 3. IR spectra of colon cancer cell in different stage of cell cycle.