Using Novel Method to Detect Different Cancer-Cell Stages of Model Human Lung Carcinoma

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Background: Synchrotron radiation infrared (SR-IR) microspectroscopy and SR-IR spectroscopic imaging are extremely valuable techniques for determining the molecular composition of biological and biomedical samples. In this work, SR-IR is applied in the study of the lung cancer cells in different cell cycles. **Methods:** We use a novel synchrotron based radiation infrared system combined synchronized model human lung carcinoma to reveal its unique character pattern. **Results:** After using SR-IR microspectroscopy, we discovered that the ratio of protein to lipid in G1 and G2 states is around 4.0 and 6.1, respectively. Moreover, for the DNA at the wavenumber position of 1225 cm^{-1} , the intensity ratio of

G2 state to G1 state is approximately 1.6. These data indicate that the cell in G1 state has more lipid composition to prepare for the DNA synthesis, but the cell in G2 state has more protein composition to prepare for the mitosis. The cell has larger DNA concentration in G2 state, which can be explained for the DNA synthesis. **Conclusion:** Through our research, we demonstrate that different growth state of cancer cell presenting unique functional groups concentration profiles and distribution via using SR-IR microspectrometry. These applications will provide another ways to improve modern cancer screening in the future. J. Clin. Lab. Anal. 29:285–288, 2015. © 2014 Wiley Periodicals, Inc.

Key words: cell cycle; lung carcinoma; microspectrometry; synchrotron; synchrotron radiation infrared

INTRODUCTION

Infrared (IR) spectroscopy using a Fourier-transform IR (FT-IR) spectrometer is a ubiquitous analytical technique nowadays. Compared with conventional IR source, higher brightness IR sources are what we need. Thus, unlike lasers, the synchrotron source is the only “white” source that delivers the entire IR wavelength range, from far-IR to near-IR (1). It is worth to note the brightness advantage of this source, which is not only that synchrotron produces more power, but also the small source size and narrow range of emission angles. IR microspectroscopy with synchrotron source has small sampling areas that can be interrogated with a superior signal-to-noise ratio using a brighter synchrotron source compared to a conventional black-body source; it also has faster acquisition time and superior spectral quality in biological study. Biosamples, comprising a mixture of many common biomolecules, such as nucleic acids, proteins, lipids, and carbohydrates, have characteristic and well-known vibrational spectroscopic in vibration, which has led to

several important and extensive clues by IR spectroscopy (2, 3). Mid-IR absorption bands are frequently observed biological samples, including amide I band at around 1650 cm^{-1} that results principally from the (C=O) stretching vibrations of protein amide bonds, amide II band close to 1549 cm^{-1} resulting from a combination of the C-N-H in-plane bending and (C-N) stretching vibrations

Additional Supporting Information may be found in the online version of this article.

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characteristic of noncyclic monosubstituted amides, the (C=O) stretching vibrations of aliphatic ester-containing lipids at about 1740 cm^{-1} , and the antisymmetric (PO_2^-) stretching of the nucleic acid backbone and phospholipids near 1225 cm^{-1} . The precise location of the characteristic bands, depending on the environment and specific variations in positions, has been clearly identified in Alzheimer's disease (4) and osteoporosis (5), as well as skin (5), breast (6), and colon cancer (7–13). In this study, we try to achieve the images of proteins and lipids with a spatial resolution in a few microns of living human carcinoma cells by synchrotron IR microspectrometry without any biochemical stains and fixatives. Also, we are going to investigate the functional groups distribution and activities profiles of living human carcinoma cells that can be identified and mapped throughout different stage.

MATERIAL AND METHODS

The chemicals such as Roswell Park Memorial Institute-1640 (RPMI-1640), penicillin-streptomycin-L-glutamine (PSG), fetal bovine serum (FBS), $1\times$ phosphate-buffered saline ($1\times$ PBS), trypsin/EDTA, L-mimosine, and nocodazole were all obtained from Sigma chemical company (St. Louis, MO). Meanwhile, A549 cells (CCL-185) were obtained from American Type Culture Collection (ATCC) as experiment model of human lung carcinoma. Low-E (SnO-coated) IR-reflecting microscope slides were obtained from Kevley Technology (Chesterland, OH). Ultrapure water ($18\text{ M}\Omega\cdot\text{cm}$), obtained by a Milli-Q purification system (Millipore, MA), was used in preparation of all the solutions. Human lung carcinoma cells, A549, were maintained in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated FBS and 1% PSG at 37°C , and 5% CO_2 in humidified atmosphere. The number of cells from the cultured solution was counted using a hemocytometer. Thereafter, the desired number of cells was concentrated with centrifuge and washed with $1\times$ PBS for further use. To synchronize A549 cells were treated with L-mimosine ($100\text{ }\mu\text{g/ml}$) or nocodazole (50 ng/ml) for 24 h to arrest cells in the G1 phase or in the G2/M phases, respectively (14, 15). Cells were released from cell-cycle blocks by washing with $1\times$ PBS and fresh medium was added. For IR analysis, cells were cultured on low-E slides. Before placing the IR slide in sterile culture dishes, the slide was cut into approximately $1\times 1\text{ cm}^2$ in size, then washed in 70% ethanol, and exposed to ultraviolet radiation overnight to sterilize IR slide. Also, the A549 cells were brought into suspension from culture flasks using trypsin/EDTA and 1×10^6 cells were seeded into each culture well. After that, the IR slide was ready to be placed in sterile culture dishes. The cells were naturally attached to the slide and were simultaneously prepared for G1/G2 phase synchronization in the culture dishes. The

Fig. 1. (A) A549 cells cultured onto pieces of IR reflective microscope slides and waited for IR analysis and (a) control cell, (b) G1 state cell, (c) G2 state cell. (B) Synchronization of A549 cells.

cell status was checked using flow cytometry technique as the standard procedure. IR spectra were recorded by end station of beamline 14A1 at National Synchrotron Radiation Research Center (NSRRC) in Taiwan. Collection was made in reflectance mode for the IR slide that was attached to the cell. Data processing was performed using the OMNIC software. A Spectra-Tech $32\times$ Refflachromat objective was used, and an aperture selected to illuminate the required sampling area, allowing spectra to be collected from single cell without interference from adjacent cells. Spectra were collected at 4 cm^{-1} spectral resolution. The sampling area and scan number of the cell in imaging were $15\times 15\text{ }\mu\text{m}$ and 128, respectively. Meanwhile, the sampling area and scan number of the cell in spectroscopy

Fig. 2. A549 cell exerts unique mid-IR spectrum profile in control, G1, and G2 growing stages.

Fig. 3. The compositions of amide and lipid in different cell cycles were integrated from the spectrum.

were $40 \times 40 \mu\text{m}$ and 2048, respectively. The set-up synchrotron IR microspectrometry detection system is in the Supporting Information Figure S1. Each measurement was performed five times and the data were calculated as mean values $\pm$ SD. The statistical validity was evaluated using student's *t*-test and *P*-values ≤ 0.001 were considered significant.

RESULTS

The Human Lung Carcinoma Model A549 Cell Line Culture in Biocompatible and Well-Synchronized IR Slides

To determine whether the noncontaminated special IR slide is a key determinant of resistance to general bio-sterile procedure, the nonviolence sterile method was used.

Before placing the IR slide in sterile culture dishes, the IR slide was cut into small pieces and exposed to UV radiation overnight, then the sterile slide was cocultured with the human carcinoma model cells A549 in culture dishes. In Figure 1A, there is no any sign of contamination, and the cells adhered to reflection side of the low-E IR slide. Furthermore, in Figure 1B indicates that the synchronization in the culture cells is successfully.

The Lung Cancer Cell Exerts Unique Mid-IR Spectrum Profile in Its Different Growing Stage Under Synchronization Medicine Treatment

Two synchronization chemical reagents have been applied in the treatment of the growing stage. One is for growing in G1 state synchronization, which is treated by $100 \mu\text{g/ml}$ of L-mimosine in culture medium. The other one is for growing in G2 state synchronization, which is treated by 50 ng/ml of nocodazole in culture medium. Both synchronization reagents were cocultured with cells for 24 h, then washed with $1 \times \text{PBS}$ before the sample was loaded on the IR station. The functional groups of the sample in different cell cycles, such as the antisymmetric PO_2^- stretching vibration characteristic of the DNA backbone ($\sim 1225 \text{ cm}^{-1}$), amide I ($\sim 1650 \text{ cm}^{-1}$), amide II ($\sim 1550 \text{ cm}^{-1}$), and ($\sim 2930 \text{ cm}^{-1}$), can be obtained from the IR spectra, shown in Figure 2. Figure 3 displays the composition of amide and lipid in different cell cycles, which is analyzed from the spectrum in Figure 2. The ratio of protein (amide I + amide II) to lipid in G1 and G2 state is around 4.0 and 6.1, respectively. Moreover, the intensity ratio of G2 state to G1 state is approximately 1.6 at the wave number of 1225 cm^{-1} for the DNA absorption. It indicates that the cell in G1 state has more lipid

Fig. 4. The IR spectrum distribution of those functional groups in difference growth stage cell.

composition in order to prepare for the DNA synthesis. On the other hand, the cell in G2 state has more protein composition in order to prepare for the mitosis. The cell has larger DNA concentration in G2 state, which can be explained for the DNA synthesis.

The Synchrotron IR Spectrum Pinpoints Mapping of Biofunctional Groups in Different Growing States of Lung Cancer Cell

The high-resolution pinpoint mappings of the distribution and frequency of lung cancer cell in difference growth stage cells, white light, and functional group of amide I, amide II and lipid mappings are displayed in Figure 4, respectively. The experimental results demonstrate that the amide I and amide II are mainly distributed in the center of the cell. However, the lipid is distributed near the cell edge. Furthermore, the frequency of amide I and II are also illustrated highly localization in the central of a cell.

DISCUSSION

In this study, we achieve the images of proteins and lipids with a spatial resolution in a few microns of living human carcinoma cells by synchrotron radiation IR (SR-IR) microspectrometry without any biochemical stains and fixatives. Also we investigate the functional groups distribution and activities profiles of living human carcinoma cells, which can be identified and mapped throughout different stages. Throughout, our experiments demonstrate that SR-IR microspectrometry can acquire highly resolved distributions and frequency of chemical groups for detecting subtle chemical differences in variable growth states of the tumor cells. SR-IR microspectrometry for cancer study may provide a new chapter to reveal the cancer behavior. In the future, the proposed idea could potentially be applied for the massive screening of many kinds of carcinomas and hopefully could prove useful for the early diagnosis, staging, and prognosis of cancer diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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