Analysis of Functional, Vibrational Components and Construction of the Imaging in Frozen-section of Cancer Model Raw Tissues by FT-IR Microscopy

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Abstract

Experimental tumor model tissue was prepared by a C6 malignant glial tumor cultivated cells to transplant under the skin of BALB-c-nu/nu strain nude mouse. The sliced tissue of the tumor model was measured by the FT-IR microscope on the SR center beam line (BL-15). The histological H.& E. stained image was compared with the mapping image of FT-IR microscopy. It was resulted that There were presented of the anti-symmetrical stretching C-H vibration components of CH_3 and CH_2 at the left-up area of the 2D FT-IRM mapping image related with the pink color part, that is necrotic area in H.& E. stained image. On the other hand, the symmetrical stretching P=O component were presented around the necrotic area as same as the viable cancer nucleus distribution in H.& E. staining image. Accordingly, these components of lipid (C-H) in the necrotic cancer area and phosphate oxide (P=O) were related in the viable cancer regions on the histo-chemical meaning of the H.& E. staining in this study.

1. Introduction

It is one of the subjects in medical science to know the differences between cancer and the normal for 140 years since H & E staining histology. It has not used to look the raw tissues of cancer in the long history. We have been observed the molecular changes of a necrotic tissue from a murine carcinoma by Fourier-transform infrared microscopy (FT-IRM)(1-3). Recently, Italian research group reported for cancer research and diagnosis using a synchrotron radiation technology in the European Community's Framework Program-Diamond Light Source (DLS) (4). We also challenged to develop of the applying technologies for the raw cancer imaging and diagnosis using the FT-IRM at the synchrotron beam line (BL-15) in SR center of Ritsumeikan University for recent three years.

2. Experimental

2.1: Preparation of the Samples of Tumor Models

Two different kinds of cultivated cancer cells, MKN-45 of human stomach cancer and C6 of rat glial tumor, were cultivated growing in CO_2 incubator (5% CO_2, temperature at 37 C) with RPMI-1640 (Code No.30263-95, Nacalai Tesque Inc., Kyoto, Japan) medium solution for 2-3 days to the full seats (2x10E6 cells/ml). The seated cells in a frasco plate were tore off the plate to suspended single cell in the new medium using a trypsin-EDTA reagent. The single cell suspension (2x10E5 cells/0.1ml) was injected under skin of the both sides on thigh of the nude BALB-c-nu/nu strain mouse. When the both tumor models were growing at about 7x7x7 (=179.50) mm^3 size, they were used for the experimental samples.

2.2: Preparation of the Samples of Frozen-section

The frozen-sectioned samples at 8-10 μm were made by the cryo-sectioning instrument (Type-CM-1950-OUVVM, Leica Biosystems Co, Ltd., Germany). The frozen-sectioned samples inside a plastic frasco dish were brought to the SR center with dry-ice.

*2.3: Measurement by the FT-IRM in SR Cente*r

The frozen-sectioned samples were put on the BaF2 plate (circle diameter:10 mm, thin:1 mm) and set into the holder fixed on the stage of the FT-IRM (Nicolet-6700 spectrometer, and Continuμm XL-type, Thermo Science Co., Ltd., USA) setting at BL-15 beamline of SR center of Ristsumeikan University. IR spectra of the raw cancer samples were measured by an individual aperture size (MKN-45 case: 7x7 μm^2; C6 case: 25x25 μm^2). These spectra were analyzed by a curve-fitting method (Voigt-function) as following conditions in Table 1.

Table 1 Curve-fitting (Voigt Function) areas

Components	Peak Position(cm^{-1})	Curve Fitting Area (cm^{-1})
CON	5156	5300-4930
C=O, OH	4601	4750-4475
Soft water	3550, 3450	3570-3470, 3470-3370
NH	3300	3350-3250
Hard water	3200, 3050	3250-3100, 3100-3000
CH_3(aSym)	2960	2970-2950
CH_2(aSym)	2925	2930-2915
CH_3(Sym)	2875	2880-2860
CH_2(Sym)	2850	2860-2820
C=O	1740	1750-1720
Amide I	1650	1700-1600
Amide II	1550	1600-1500
	1450	1470-1420
	1390	1410-1370
	1300	1350-1250
PO(aSym), (Amide III)	1230	1260-1200
PO(Sym)	1080	1100-1050
	1030	1050-1000
P-O-P	970、920	1000-950, 950-900

3. **Results and Discussion**

3.1: MKN-45 Stomach Cancer Model

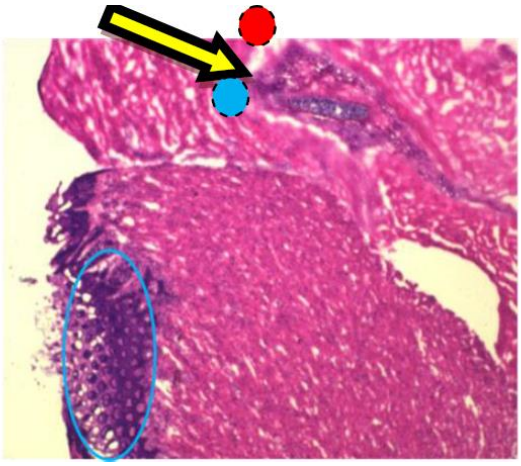

Fig.1: H.& E.-staining of MKN Tissue.

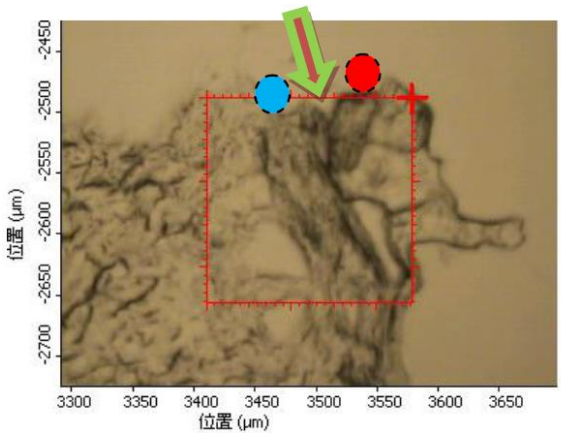

Fig. 2: Transmitted Image of MKN Slice.

Cancer region was focused as shown by the arrow in Figure 1. The transmitted image (red square) in Figure 2 corresponds to the H & E stained histological image. The blue color stained area with H & E dyes (in the left hand side of the arrow) was cancer region. There are also cancer regions both sides on the way of the arrow as shown in Figures 1 and 2.

Figure 3 shows a FT-IR spectrum taken at the point (tip) of the arrow as shown in Figure 2. We focused on 3 peaks as shown in the spectrum. The peak area was analyzed by the curve-fitting (Voigt Function which was explained in Table 1) to show the mapping images in Figures 4 (aSym: asymmetrical CH_2, 2925 cm^{-1}), 5 (ester C=O, 1740 cm^{-1}), and 6 (Sym: symmetrical PO_2, 1230 cm^{-1}), respectively. These 3 components of stretching vibration in the cancer region were nice collation with each other as shown in these mapping images (Figures 4, 5 and 6).

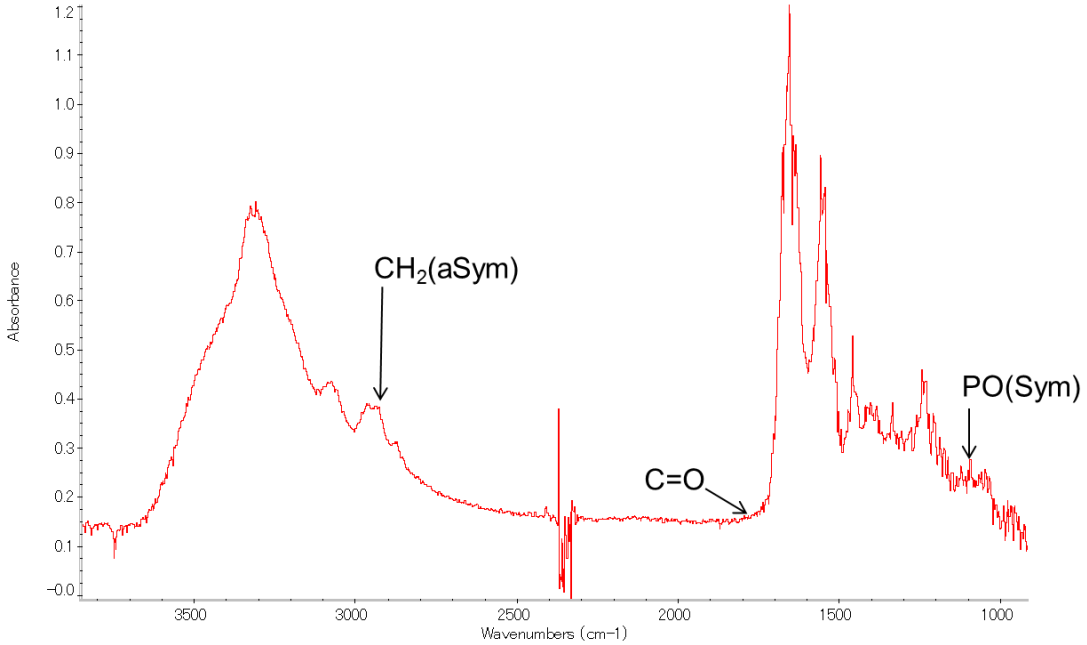

Fig.3: A FT-IRM spectrum of a MKN slice.

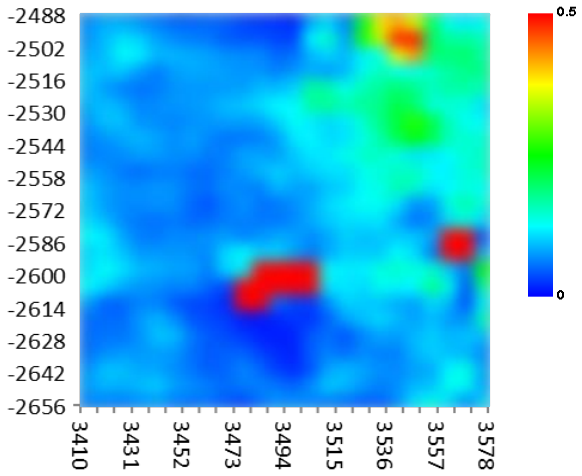

Fig.4: Mapping of aSym CH_2.

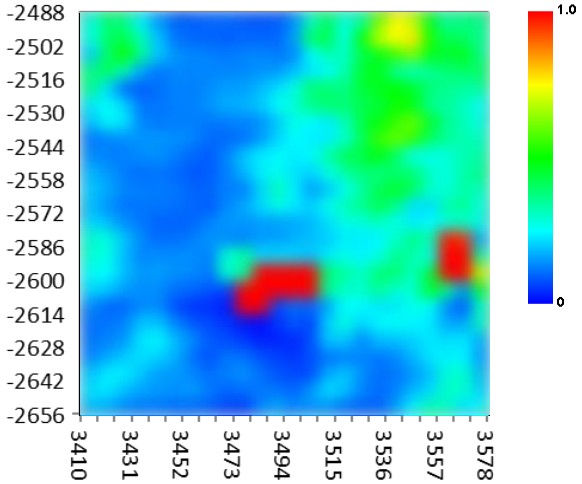

Fig.5: Mapping of C=O.

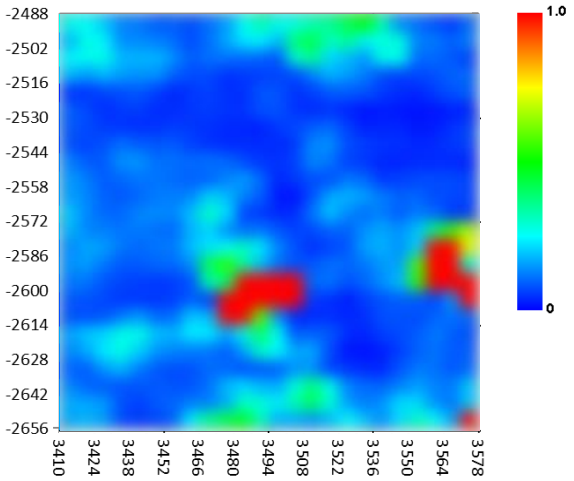

Fig.6: Mapping Sym PO_2.

3.2: C6 Glial (Brain) Tumor Model

On the other tumor model tissue, C6 glial tumor, was observed by the same mapping methods with the FT-IRM in the case of MKN-45 stomach cancer tissue (*3.1*) as following sample area in Figures 7. There was presented C6 glial tumor tissue under the normal skin

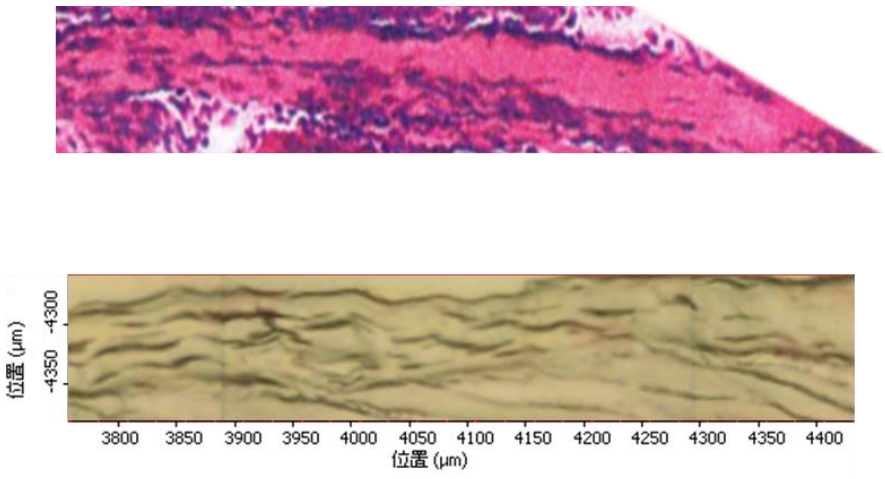

Fig.7: The H & E stained (upper) and the transmitted (lower) images of C6 glial cancer tissue.

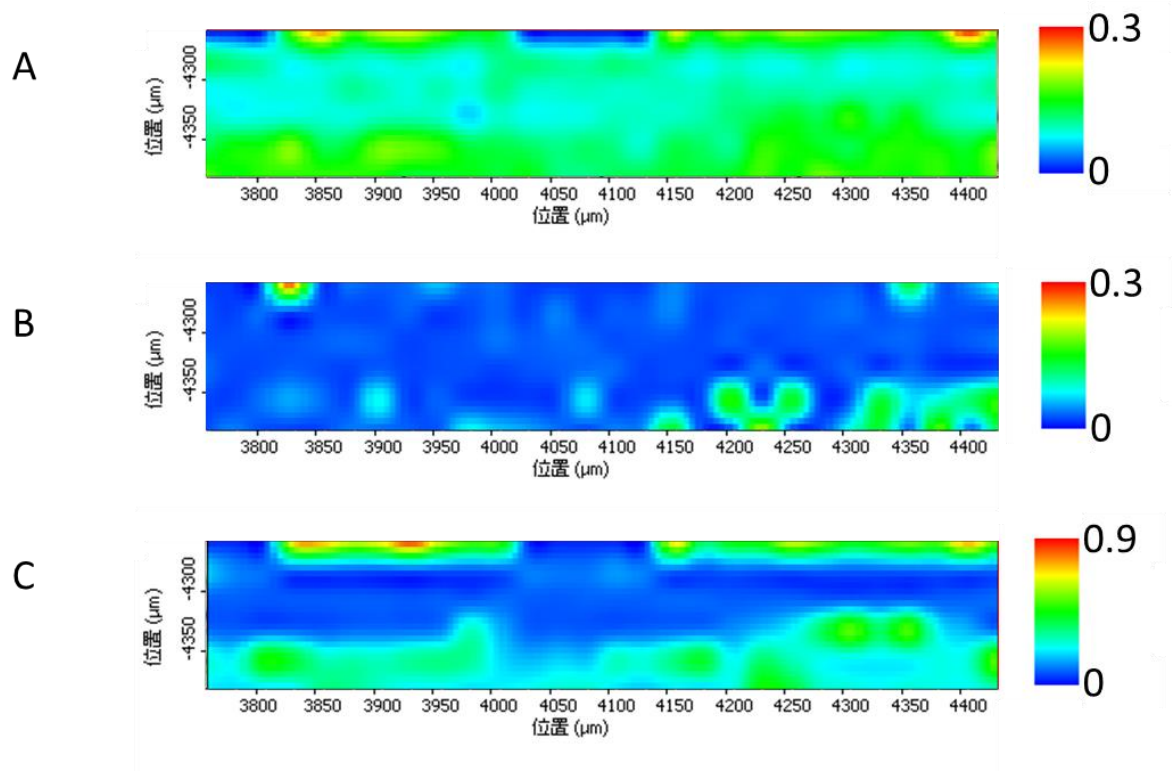

Fig.8: The mapping images at 2925 cm^{-1} (SV-aSym CH_2) [A], 1740 cm^{-1} (C=O) [B], and 1080 cm^{-1} (SV-Sym PO_2) [C], respectively.

one as shown in the H & E stained and the transmitted images. In the C6 cancer region, we have researched what kinds of vibrational components were dominant. The result was that the dominant vibrational components in cancer region were asymmetrical stretching vibrational (SV-aSym) of C-H in CH_2, ester C=O, and symmetrical stretching vibrational (SV-Sym) of PO_2, respectively as shown in Figure 8.

4. Conclusions

It was studied what kinds of vibrational components were dominant in cancer region. As a result, there was presented the components of asymmetrical stretching vibrational of C-H inCH_2, ester C=O, and symmetrical stretching vibrational of PO_2 in cancer region. From these facts by FT-IRM mapping method, it was reasonable to be large amount of DNA contents in cancer, which means including large amount of phosphate as the bone of the DNA in the cancer large nucleus. Furthermore, it was found that the lipid components of ester (C=O) and C-H of CH_2 was dominant in cancer region, which means the necrotic region in cancer tissue also presents in the advanced cancer region as a histological specific

point. These findings in FT-IRM measurements will change the old histological practical senses gradually in future.

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