Differences between infrared spectra of normal and neoplastic human gastric cells

Naoko Fujioka^a, Yuji Morimoto^{a,*}, Tsunenori Arai^b, Kyoko Takeuchi^c, Masahiko Yoshioka^c and Makoto Kikuchi^a

^a *Department of Medical Engineering, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan*

^b *Department of Applied Physics and Physico-Informatics, the Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan*

^c *First Department of Anatomy, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan*

Abstract. We investigated the differences in Fourier transform-infrared (FT-IR) spectra between normal and neoplastic human gastric cells. The infrared spectra derived from the cancer cells (AGS, SNU-1, and NCI-N87) showed a significant increase in infrared absorption in the band around 1240 cm^{-1} , 1120 cm^{-1} and 1080 cm^{-1} , compared with those from the normal gastric epithelial cells we established in culture. In addition, frequency shifts of 4.6 cm^{-1} and 3.8 cm^{-1} were seen at the peak absorbance in the bands around 1240 cm^{-1} and 1080 cm^{-1} , respectively. These spectral differences reflected the differences between the phosphate backbone in the normal and neoplastic human gastric cells. The present results suggest that FT-IR spectroscopy is a potential new tool for gastric cancer diagnosis.

1. Introduction

Gastric cancer is a leading cause of cancer death worldwide. Although its incidence has steadily decreased over the last six decades in the United States and Western Europe, gastric cancer remains the most common malignancy for both sexes in Asia and Latin America [1]. To reduce the incidence of this cancer, many efforts have been made to improve early diagnostic and therapeutic procedures.

Fourier transform infrared (FT-IR) spectroscopy is a sensitive analytical technique with practical advantages. It requires only small samples and allows non-destructive analysis of structural and chemical/physical properties of samples at the molecular level [2,3]. Recently, FT-IR spectroscopy has been extensively used as a cancer diagnostic technique because of its potential for revealing biological changes in diseased cells in the process of carcinogenesis [4–7]. Cohenford et al. showed that the FT-IR spectra of pelleted exfoliated cells from patients with cervical cancer or dysplasia differed from those of such cells from normal women [4]. In addition, differences between FT-IR spectra of colon adenocarcinoma cells and normal colon tissues have been reported [8]. However, there have been no reports comparing FT-IR spectra of normal and neoplastic human gastric cells. Therefore, in the present study, we investigated the differences between FT-IR spectra of normal gastric cells and gastric cancer cells to clarify the IR spectral features of gastric cancer cells. For this purpose, we have established normal gastric epithelial cell lines and used these cells as the control for comparison with gastric cancer cells. Herein we show

*Corresponding author: Yuji Morimoto, MD, PhD, Department of Medical Engineering, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. Fax: +81 42 996 5199; E-mail: moyan@cc.ndmc.ac.jp.

the essential difference in FT-IR spectra between normal and neoplastic human gastric cells, indicating the great potential of FT-IR as a practical diagnostic tool for gastric cancer.

2. Materials and methods

2.1. Gastric mucosal epithelial cells

Human gastric mucosal epithelial cells were prepared using a modified method of Wagner et al. [9], and initiation of the primary culture was based on the methods of Bagchi et al. [10] and Basque et al. [11]. Normal stomach tissues were obtained from 8 randomly selected patients (mean age, 62 y/o, ranging from 48 to 80 y/o) undergoing gastrectomy at the National Defense Medical College. The appropriate ethics committees approved the study, and all patients gave their informed consent. Surgical specimens (4–6 cm²) were taken from a pathologically healthy part of the body and antrum region of the removed stomach. The tissue specimens were chopped into small cubes (3 × 3 × 3 mm³) and both the mucosal epithelium and the lamina propria mucosae were sliced off from the cubic samples. The sliced specimens were centrifuged at 50–100g for 5 min and the resulting pellets were stirred in phosphate buffered saline (PBS) containing 0.2% (w/v) collagenase type I (Wako, Osaka, Japan) and 1% (w/v) DNase I (Roche Diagnostics GmbH, Mannheim, Germany) for a few seconds. They were then incubated to dissociate the cells for 15 min, and the dissociated cells were filtered through a nylon mesh (pore = 40 μm) and collected and centrifuged at 50–100g for 10 min. After discarding the supernatant PBS, the cells were resuspended and re-centrifuged. The resulting pellets were seeded in a collagen-coated plastic culture dish with a 5-ml medium (Keratinocyte-SFM, Gibco, Invitrogen, Carlsbad, CA) containing penicillin (100 U/ml), streptomycin (50 μg/ml), and 10% fetal bovine serum (Gibco). The seeded cells were incubated at 37°C for 24 h in a humidified incubator in a 5% CO₂ atmosphere. When the cells reached confluence, the cells were passed by harvesting with 0.25% trypsin-1 mM EDTA (Gibco) at 37°C and seeded. In order to maintain pure culture, we removed fibroblastic cells from the dishes using a silicon rubber scraper every 48 h. For the FT-IR experiments, the primary culture cells were used.

To confirm whether the cultured cells had the characteristics of normal gastric mucosal epithelium, they were examined histochemically and morphologically. Expression of protein markers, including pepsin (Anti-Pepsin, Polysciences, Washington, PA), gastric mucin (MUC5AC, Labvision, Fremont, CA) and gastric H⁺/K⁺-ATPase (Anti-H⁺/K⁺-ATPase (β-subunit), Affinity Bioreagents, Golden, CO) was detected as described previously [10,11]. For ultrastructural analysis, the cultured cells were fixed with 2.5% glutaraldehyde-PBS for 1 h and then washed with PBS. They were postfixated in 1% OsO₄–100 mM phosphate buffer (pH 7.4), dehydrated, and embedded in Epon. The horizontal ultrathin sections were examined by electron microscope.

2.2. Gastric cancer cell lines

Three human gastric adenocarcinoma cell lines, AGS (differentiation grade not known), SNU-1 (poorly differentiated), and NCI-N87 (well differentiated) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). In the FT-IR experiment, cells were used at the 5th passage. The three cell lines were cultured by standard methodology and according to the specific instructions of the ATCC.

2.3. FT-IR measurement

Confluent cells were harvested with 0.25% trypsin-1 mM EDTA. The following preparation of cellular pellets was carried out at 4°C. The harvested cells were pelleted by centrifugation and a small amount (almost 0.01 mg) of the cell pellets was placed on a diamond window (Diamond Window™, $\phi = 3$ mm, Sumitomo Electric, Tokyo, Japan). The pellets were air-dried for 3 min under atmospheric humidity <60% [12]. The IR absorption spectrum of each pellet was obtained with a Fourier transform infrared spectrometer (FT/IR-620, Jasco, Tokyo, Japan) with a resolution of 2.5 cm^{-1} . The size of the aperture ($100 \times 100\text{ }\mu\text{m}$) allowed IR spectral measurement of 25~100 gastric cells each time. In order to exclude a masking effect due to thickness of the sample, each IR spectrum was normalized by scaling the entire spectrum to the IR absorbance at 1650 cm^{-1} (amide I), thus showing that the IR absorbance at 1650 cm^{-1} was 100% absorbance.

2.4. Statistical analysis for FT-IR spectra

The frequency region of $1650\text{--}925\text{ cm}^{-1}$ was chosen for the analysis because previous studies have shown many differences between normal and cancerous cells in that region [4,6]. At each spectral wavenumber, a *t*-test for the difference between absorbances of normal cells and cancer cells was conducted, yielding one *P* value per frequency.

3. Results

3.1. Characteristics of gastric mucosal epithelial cells

In the course of primary culture, we confirmed that the transferred cells first adhered to each other, and then began to spread, finally forming a monolayer of dense epithelial colonies. Individual cells displayed a polyhedral epithelial morphology. Tight junctions were present on the free surface (Fig. 1A). Numerous normal structural mitochondria were seen, in which the crista consisted of the inner mitochondrial membrane (Fig. 1B). Immunohistochemical examination revealed that every cell in the growing colonies

Fig. 1. Electron photomicrograph of gastric epithelial cells in primary cultures. Note the desmosome in the intracellular space (A, ▲ ▲) and the many normal mitochondria in the cytoplasm (B). Bar = 200 nm (A), and 500 nm (B).

expressed either mucin, pepsin, or gastric proton pump ($H^+/K^+-ATPase$). These results indicated that these cultured cells originated from either surface mucinous cells/superficial epithelial cells, chief cells, or parietal cells. The integrity of the confluent monolayer remained unaltered for at least 2 months. Obvious differences among 8 cultured cells, each of which was obtained from a different patient, were not seen morphologically or histochemically.

3.2. Features of IR absorption spectra of normal cells and cancer cells

Figure 2A shows the mean IR absorbencies ($1650-925\text{ cm}^{-1}$) of the normal gastric mucosal epithelial cells and three types of cancer cells. The gross spectral features of the IR spectra mostly resemble those

Fig. 2. A, IR absorbance spectra of normal mucosal epithelial cells and three types of gastric cancer cells. In the normal cells, spectrum represents a mean absorbance of 8 measurements, each of which was obtained from a different patient. In the cancer cells, spectrum represents a mean absorbance of 14, 7, and 5 measurements in AGS, SNU-1, and NCI-87, respectively. Each measurement of the cancer cells was obtained from a different cell sample. Each spectrum was normalized by scaling the entire spectrum to the IR absorbance at 1650 cm^{-1} (amide I). Asterisks indicate absorption bands. B, Statistical significance in IR absorbance of the normal cells versus each type of cancer cells, based on a *t*-test with unequal variances. Note the prominent differences in IR absorbance in the bands around 1240 , 1120 and 1080 cm^{-1} (dotted lines parallel to *y*-axis).

of other organ cells previously reported [4–7]. Figure 2B shows the P values associated with tests for differences in absorbance between normal cells and cancer cells at each wavenumber. A P value above 0.05 suggested that there was no significant difference in absorbance between normal and cancer cells, whereas a P value below 0.05 indicated a significant difference. Eleven absorption bands (asterisks) were seen in the frequency range ($1650\text{--}925\text{ cm}^{-1}$). Three bands out of the eleven bands showed notably small P values (<0.01) in any comparison tests (dotted lines parallel to y -axis). The three bands included a band having the maximum peak around 1240 , 1120 , and 1080 cm^{-1} .

3.3. IR absorptions at 1240 cm^{-1} , 1120 cm^{-1} and 1080 cm^{-1}

At the three wavenumbers (1240 , 1120 , and 1080 cm^{-1}), IR absorbance values in any cancer cells were greater than those of normal cells (Fig. 3). In addition, significant differences were seen among the IR absorbance values of the three types of cancer cells. Apart from absorbance values, a significant frequency shift was seen in the bands around 1240 and 1080 cm^{-1} (Fig. 4). Around 1240 cm^{-1} , peak maximum absorbance in normal cells was observed at 1236.9 cm^{-1} (mean) while cancer cells (AGS, SNU-1 and NCI-N87) showed peak maximum absorbance at 1241.5 cm^{-1} (mean), indicating a 4.6-cm^{-1} shift accompanying malignant alteration (Fig. 4). Similarly, peak maximum absorbance in the band around 1080 cm^{-1} shifted from 1081.9 cm^{-1} (normal) to 1085.7 cm^{-1} (AGS and SNU-1) as Fig. 4 shows. On the other hand, the peak appeared in NCI-N87 cells was observed at 1081.5 cm^{-1} (mean), indicating a -0.4-cm^{-1} shift when compared with that in normal cells (Fig. 4). However, the -0.4-cm^{-1} band shift was not significant since the spectral resolution in the present study was 2.5 cm^{-1} , as described in the methods. Peak maximum absorbance in the band around 1120 cm^{-1} was not clear in the normal cells or AGS cells.

Fig. 3. IR absorbance of normal mucosal epithelial cells and gastric cancer cells, including AGS, SNU-1, and NCI-87 at the peak maximum in the band around 1240 cm^{-1} , 1120 cm^{-1} and 1080 cm^{-1} . IR absorbances of any types of cancer cells are greater than those of normal cells in all three frequency bands. Each absorbance was normalized to the absorbance at 1650 cm^{-1} . Bars indicate means.

Fig. 4. Wavenumber at peak maximum absorbance in the band around 1240 cm⁻¹ and 1080 cm⁻¹. Around 1240 cm⁻¹, wavenumber at peak maximum absorbance in normal cells (1236.9 cm⁻¹) is significantly smaller than those in any cancer cells (AGS, 1240.7 cm⁻¹; SNU-1, 1241.9 cm⁻¹; NCI-N87, 1243.1 cm⁻¹). In the band around 1080 cm⁻¹, wavenumber at peak maximum absorbance in the two types of cancer cells (AGS and SNU-1) also shifts when compared with that in normal cells.

4. Discussion

4.1. IR absorption in the bands around 1240 cm⁻¹, 1120 cm⁻¹ and 1080 cm⁻¹

Three remarkable spectroscopic features were confirmed in human gastric cancer cells in the present study. First, IR absorption in the bands around 1240 cm⁻¹ and 1080 cm⁻¹ increased in these cells. Since these bands were previously identified to be the vibration mode of PO₂⁻ of the phosphodiester groups [5], the increased IR absorption in these bands suggests an increase in nucleic acids [13]. Second, in the 1080-cm⁻¹ band, the absorption peak maximum appearing at 1081.9 cm⁻¹ in the normal cells was shifted to 1085.7 cm⁻¹ in two types out of three types of cancer cells. This shift can be attributable to the tighter packing of DNA molecules in the abnormal cells [6,14,15]. Apart from the 1080-cm⁻¹ band, the peak maximum in the band around 1240 cm⁻¹ moved toward a higher frequency in the cancer cells, suggesting a change in hydrogen bonds in the phosphodiester groups of the DNA molecules in abnormal gastric cells [7,14]. Third, a significant increase in absorbance was seen in the shoulder band at 1120 cm⁻¹ in the cancer cells. This band is due to increase in RNA contents in the cancer cells [5,16]. As explained, these three remarkable features are all related to an increase in the phosphate backbone. This increase in the phosphate backbone may correspond to increase in DNA and RNA content of the cancer cells.

4.2. Application and limitation of FT-IR spectroscopy

FT-IR makes it relatively easy to identify structural and chemical/physical properties at the molecular level. Thus, this method has been clinically employed to detect or classify cervical lesions. For the diagnosis of cervical cancer, the sensitivity has reached 79% when FT-IR is applied [10]. The limitations

of the FT-IR method include the difficulty in measuring thick samples and water-rich samples. Nevertheless, FT-IR is a promising tool for cancer diagnosis because of the rapid processing time and the ease of operation, without the need for specialists. This method can be applied to exfoliative cells and small tissue obtained by biopsy. Our results strongly suggest that FT-IR spectroscopy is a useful technique for screening gastric cancer.

5. Conclusion

In the present study using FT-IR spectroscopy, we demonstrated the differences between infrared spectra of normal gastric epithelial cells and gastric cancer cells. Since the control IR absorption spectra were derived from the cultured normal gastric mucosal cells, the differences in IR absorbance between the normal gastric cells and cancer cells may, for the most part, reflect a purely biochemical difference in intra-cellular components as well as in the cellular membrane. These spectral differences were seen substantially in the bands around 1240 cm^{-1} , 1120 cm^{-1} and 1080 cm^{-1} .

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