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Original Article

Common Raman Spectral Markers among Different Tissues for Cancer Detection

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Abstract

Introduction
Raman spectroscopy is a vibrational spectroscopic technique, based on inelastic scattering of monochromatic light. This technique can provide valuable information about biomolecular changes, associated with neoplastic transformation. The purpose of this study was to find Raman spectral markers for distinguishing normal samples from cancerous ones in different tissues.
Materials and Methods
Ten tissue samples from the breast, colon, pancreas, and thyroid were collected. A Raman system was used for Raman spectroscopic measurement of tissues at 532 nm laser excitation. Five to six Raman spectra were acquired from each sample (a total of 52 spectra). Raman spectra were investigated in important bands associated with Amid1, CH2 (scissoring), Amid3, δ(NH), ν(C-C), and δ_{as} (CH3) in both normal and cancerous groups. In addition, common spectral markers, which discriminated between normal and cancerous samples in the above tissues, were investigated.
Results
Common spectral markers among different tissues included intensities of Amid3 and CH2 (scissoring) and intensity ratios of I(Amid1)/I(CH2), I(ν(C-C))/I(CH2), and I(δ(NH))/I(CH2). This study showed that Amid1-, ν(C-C)-, and δ(NH)-to-CH_2 intensity ratios can discriminate between normal and cancerous samples, with an accuracy of 84.6%, 82.7%, and 82.7% in all studied tissues, respectively.
Conclusion
This study demonstrates the presence of common spectral markers, associated with neoplastic changes, among different tissues.
Keywords: Breast cancer; cancer detection; colon cancer; pancreas cancer; Raman spectroscopy; thyroid cancer.

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1. Introduction

The term 'neoplasia' refers to tissue growth, independent of and faster than normal tissue development. A neoplasm (tumor) may be categorized as benign or malignant. Malignant tumors are also known as cancer, mostly originating from a single cell. This single cell can be converted to a group of neoplastic cells, which are distinguished by increased nuclear material, elevated nuclear-to-cytoplasm ratio, increased mitotic activity, abnormal chromatin distribution, and decreased differentiation [1]. These general characteristics of neoplastic cells cause particular changes in the quantity and/or conformation of nucleic acids, proteins, lipids, and carbohydrates.

There are several molecular markers in the cell membrane, cytoplasm, nucleus, and extracellular space, which are indicators of neoplasia. These marker molecules are proteins, lipids, and nucleic acids. In neoplastic cells, either the marker itself or its interaction with another molecule undergoes noticeable changes [2]. Cellular or molecular changes may result in Raman spectra, which are different between [2-5].

for cancer detection in breast [6-11], colon [12], gastric [13, 14], and gynecologic [15-18] tissues. A variety of different methods for sample preparation and spectra acquisition/processing have been employed in these studies [19, 20]; the obtained results have yielded different features for cancer detection in each tissue. Some of these features are common among different tissues. For instance, in a study by Feld et al., increased intensity of nuclear band and decreased lipid band intensity were reported in cancerous and normal colon and bladder samples, respectively [21].

In addition, increased protein to lipid content in neoplastic tissues has been reported in some previous studies, which were separately performed on different tissues [22]. In particular, the ratio of 1655 cm^{-1} (Amid1) band to 1450 cm^{-1} (CH_2) band has been separately used for discrimination between normal and cancerous bending mode samples in different tissues [2].

Despite the variation of cancer markers in different tissues, existence of some similarities in cancer developing mechanism and Raman markers of different cancers increases the possibility of finding common spectral Raman markers among different tissues. By accomplishing this purpose and detecting cancers independent of tissue type, it will be possible to detect cancers of unknown origin, which occur due to cell migration to other tissues (metastasis).

Considering the abovementioned points, the objective of this study was to find common spectral markers among breast, colon, pancreas, and thyroid tissues to demonstrate that evaluations can be further extended to other tissues and cancerous forms with unknown origins.

2. Materials and Methods

Normal and cancerous samples of breast (invasive ductal carcinoma), colon (adenocarcinoma), pancreas (adenocarcinoma), and thyroid (papillary carcinoma) were collected from five patients, undergoing biopsies due to clinically suspicious lesions. The minimum size of samples was 2cm×2cm×0.5cm, meeting the semi-infinite tissue geometry model [3], with respect to the optical properties of tissues at excitation laser wavelength of 532nm (resembling in vivo conditions) [23-25].

This study was approved by the ethics committee of Tarbiat Modares University, Tehran, Iran. After performing biopsies, tissue samples were fixed in formalin solution and transferred to a laboratory for Raman measurements. Five to six Raman spectra were acquired from different points of each sample (a total of 10 samples taken out of formalin); the total number of spectra amounted to 52spectra. The detailed statistics of measured spectra per tissue type and status are reported in Table 1.

Table 1. Number of collected spectra per tissue type and status

	Breast	Colon	Pancreas	Thyroid	Total
Normal	10	5	5	5	25
Cancerous	11	5	5	6	27
Total	21	10	10	11	52

Raman measurements were performed with Thermo Nicolet Almega micro-Raman system. This system uses a Nd:YLF laser that provides an excitation wavelength of 532 nm and power of 50mW. The scattered Raman signal was integrated for 5 s in backscattering geometry after 32 scans in a spectral range of 400-4000cm^{-1} with a resolution of 4cm^{-1}.

The "gold standard" for detection of malignancies was excisional biopsy, followed by histopathological analysis. After Raman measurements, the tissue samples were transferred back to the hospital for histopathological examinations. Histopathology relies upon sectioning the tissue with a thickness of <10 µm, staining with haematoxylin and eosin (H&E), and viewing under a conventional light microscope [26]. After performing the analysis, based on the evaluation of morphological and structural patterns, a label (normal or cancerous) was assigned to each sample.

2.1. Processing

The raw spectra obtained from tissue samples are a combination of major tissue auto-fluorescence, weak tissue Raman scattering signal, and noise [13]. Auto-fluorescence background was eliminated using the fifth order polynomial fitting and subtracting the fitted polynomial from the raw spectrum.

For noise reduction, the spectra were processed, using a second-order Savitzky-Golay filter. In addition, each spectrum was normalized, using the standard normal variate (SNV) procedure, as mentioned below:

$$\mathbf{y}_{SNV} = \frac{\mathbf{y} - mean(\mathbf{y})}{std(\mathbf{y})} \qquad (1)$$

where $\mathbf{y}$ is the spectrum, $mean(\mathbf{y})$ is the average of all spectral points, and $std(\mathbf{y})$ is the standard deviation of spectral points. Then, the minimum of each spectrum was subtracted from the spectrum itself to obtain non-negative spectra that would be appropriate for band intensity comparison.

To compare the Raman spectra of cancerous and normal tissues, we plotted the mean normalized Raman spectra of normal and cancerous samples of each tissue (Figure 1).

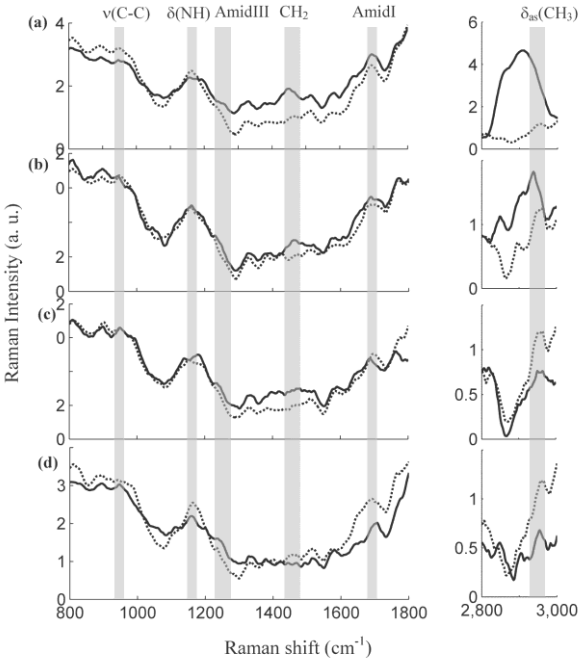

Figure 1. Mean normalized Raman spectra of normal (solid line) and cancerous (dotted line) samples of a) breast, b) colon, c) pancreas, and d) thyroid

Prominent Raman peaks, which can be attributed to Amid1, CH_2 (scissoring), Amid3, δ(NH), ν(C-C), and $\delta_{as}(CH_3)$, were observed in both normal and cancerous samples of all tissues (Table 2)-it should be mentioned that ν, δ and δ_{as} refer to stretching mode, bending mode and asymmetric bending mode respectively. There are obvious changes in Raman peak intensities between normal and cancerous samples of different tissues, which demonstrate the viability of Raman spectroscopy for cancer detection in different tissues.

Table 2. Peak assignment for Raman tissue spectra

Assignments	Peak position (cm^{-1})
ν(C-C) of proteins	928-940
δ(NH) of proteins	1147-1271
Amid III of proteins	1230-1300
CH_2(scissoring) of lipid/proteins	1420-1450
Amid I of proteins	1640-1680
$\delta_{as}(CH_3)$ of proteins	2918

ν: stretching mode, δ: bending mode, δ_{as}: asymmetric

3. Results

When comparing the six Raman peak intensities (Table 2) in the mean spectra of normal and cancerous samples of each tissue (Figure 1), it could be observed that the intensity of Amid3 and CH_2 (scissoring) decreased in all cancerous samples, compared to those in normal samples. However, other bands did not show the same trend in the tissues. Therefore, considering the mean spectra of samples, by using these two bands, we could better differentiate between normal and cancerous samples in different tissues.

Furthermore, we plotted the box charts of significant Raman peak intensities, correlating with their histopathological labels, and searched for the best separation value between the two groups in the box chart of each Raman intensity (Figure 2). As we expected, Amid3 and CH_2 bands could better discriminate normal tissues from cancerous ones with an accuracy of 75%, sensitivity of 74%, and specificity of 76% (Table 3). A scatter plot that combined these two peak intensities for both pathological types showed distinct clusters and consequently a good discrimination between the two pathological groups (Figure 4a).

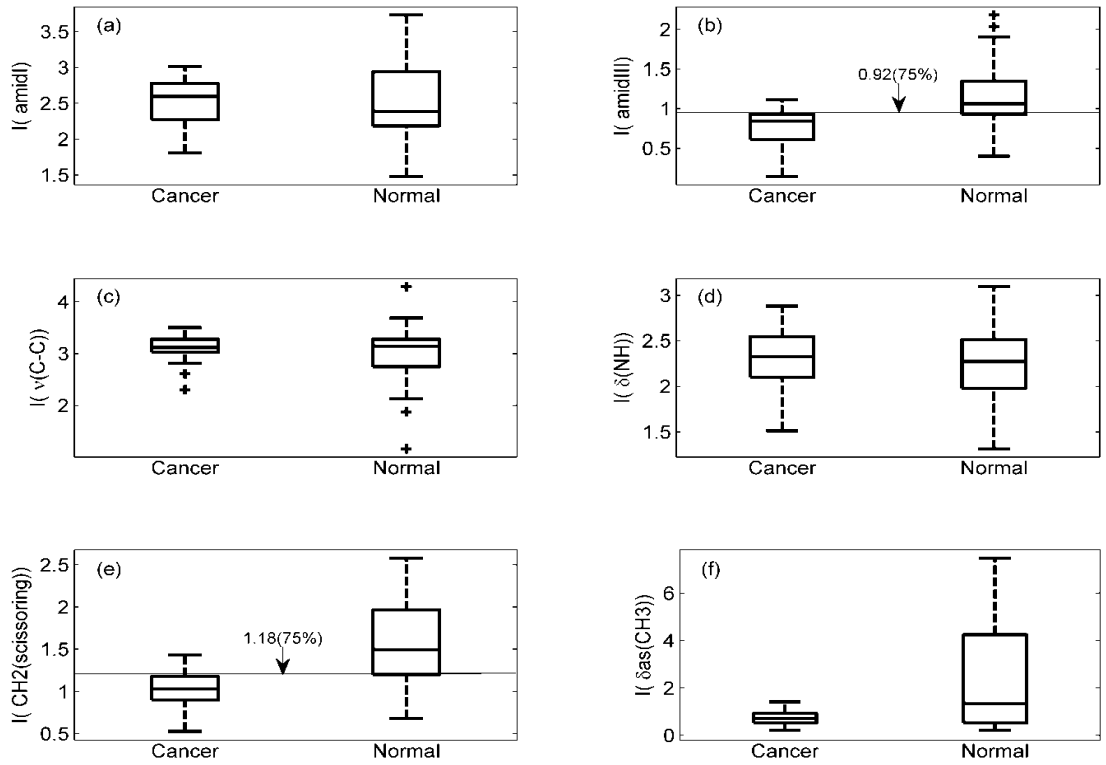

Figure 2. Box charts of six significant Raman peak intensities with their histopathological labels

Table 3. Results of discrimination between cancerous and normal tissues, using absolute and relative intensities

	Absolute intensity		Relative intensity		
	$I(CH_2)$	I(Amid III)	$I(AmidI)/I(CH_2)$	$I(\nu(C\text{-}C))/I(CH_2)$	$I(\delta(NH))/I(CH_2)$
Accuracy	0.75(39/52)	0.75(39/52)	0.846(45/52)	0.827(43/52)	0.827(43/52)
Sensitivity	0.74(20/27)	0.74(20/27)	0.852(23/27)	0.889(24/27)	0.852(23/27)
Specificity	0.76(19/25)	0.76(19/25)	0.84(21/25)	0.76(19/25)	0.8(20/25)

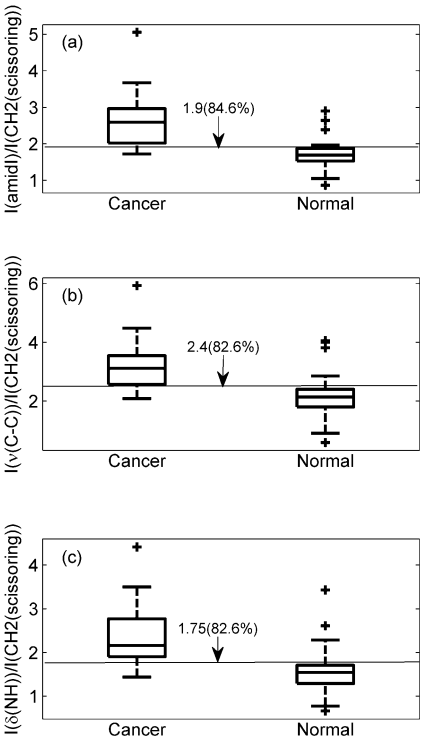

Figure 3: Box charts of a) I_{Amid1}/I_{CH2}, b) $I_{\nu(C\text{-}C)}/I_{CH2}$, and c) $I_{\delta(NH)}/I_{CH2}$ in normal and cancerous samples

Although the use of absolute intensities can yield satisfactory results, it is not entirely reliable in Raman spectroscopy and can be affected by measurement conditions. Intensity ratios as diagnostic algorithms are advantageous since they are inherently independent of Raman spectroscopic measurement conditions such as excitation/detection geometries, power fluctuations of excitation light, and probe-tissue positioning variations [13]. Therefore, we studied the intensity ratios of all six bands, relative to each other (15 states), in two normal and cancerous tissue groups, using the box chart method.

Among all intensity ratios, the intensity ratios of Amid1-, ν(C-C)-, and δ(NH)-to-CH_2 could better discriminate between normal and cancerous tissues with an accuracy of 84.6%, 82.7%, and 82.7%, sensitivity of 85.2%, 88.9%, and 85.2%, and specificity of 84%, 76%, and 80%, respectively (Figure 3, Table 3). The potential of using a pairwise combination of these three intensity ratios, as discriminating features, between the two pathological groups was illustrated, using scatter plots. Figures 4.b-4.d show well-differentiated clusters for normal and cancerous tissues.

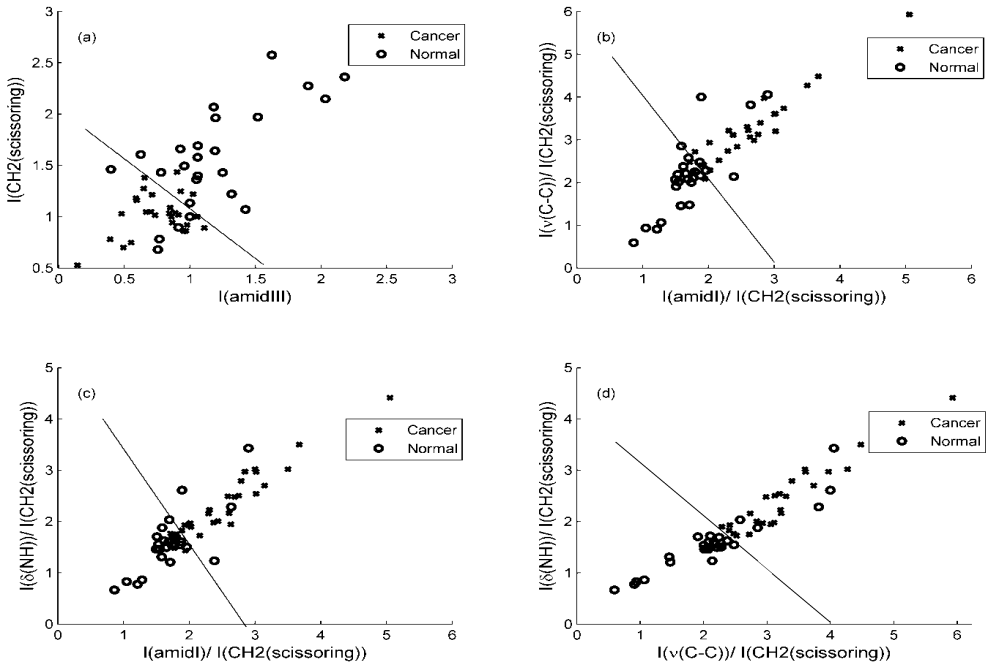

Figure 4. Scatter plots of a) I_{CH2} and $I_{AmidIII}$; b) $I_{\nu(C-C)}/I_{CH2}$ and I_{AmidI}/I_{CH2}; c) $I_{\delta(NH)}/I_{CH2}$ and I_{AmidI}/I_{CH2}; and d) $I_{\delta(NH)}/I_{CH2}$ and $I_{\nu(C-C)}/I_{CH2}$ in normal and cancerous samples

4. Discussion

Considering the similarities between different tissues in terms of cancer origination and progression, common cancer markers were expected to be found among different tissues. This study, for the first time, investigated common cancer markers among different tissues. These markers could be useful for detection of cancers with unknown origins or determination of suspicious lesions, which appear in a tissue other than the original tissue due to cell migration (metastasis).

Therefore, we used Raman spectroscopy for finding these common cancer markers among four different tissues. As the tissue type (breast, colon, pancreas, and thyroid) in this study was irrelevant to the process of finding common spectral markers, the findings can be used for the detection of cancers with unknown origins.

This study shows that there are common distinctive spectral features between normal and cancerous samples in different tissues. For instance, the intensity of Amid3 (of proteins) and CH_2 (of proteins and lipids) in cancerous samples decreased, compared to normal samples in all the studied tissues. This is most likely due to the decreased protein and lipid content in cancerous tissues, compared to normal samples.

Investigation of intensity ratios illustrated that the ratios of Amid1-, ν(C-C)-, and δ(NH)-to-CH_2 in cancerous tissues were greater than those in normal tissues; this may be due to the increase of protein-to-lipid ratio in cancerous tissues. This increased change of content (protein to lipid) in neoplastic tissues has been reported in some previous studies, which were separately performed on different tissues [26]. Specifically, the ratio of 1655 cm^{-1}(Amid1) band to 1450 cm^{-1} (CH_2) band has been used for discrimination between normal and cancerous tissues [2].

In this study, investigation of intensity ratios showed that the ratio of three prominent band intensities to the intensity of CH_2 (1450 cm^{-1}) band could be a good discriminator between cancerous and normal tissues. In some other studies, the band at 1450 cm^{-1} was shown to have the same importance for cancer detection [2, 3, 13, 25]. Therefore, it can be deduced that this band (1450 cm^{-1}) can be proposed as a standard candidate for cancer detection.

To confirm the obtained results and their application for other tissues, future studies on other tissues, with a larger number of samples, may be necessary. In addition, special feature selection algorithms can be investigated to determine optimal features for tissue classification.

5. Conclusion

A review of previously published studies revealed some similarities in Raman markers of different cancers. In the current study, we investigated the existence of common Raman cancer markers among different tissues and found three common Raman features, related to different studied cancers.

Considering the fact that the type of tissue was irrelevant to the process of finding common spectral markers in our study, application of these markers could be suggested for detection of cancers with unknown origins (or metastatic cancers), though further research is required.

According to previous studies, differences between the Raman spectra of fresh and fixed tissues are insignificant and do not affect the potential capability of the spectrum [3]. Therefore, the results of this study may be also extended to in vivo applications.

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