Journal of Clinical Laser Medicine & Surgery
Volume 19, Number 1, 2001
Mary Ann Liebert, Inc.
Pp. 35–39

UV Reflectance Spectroscopy Probes DNA and Protein Changes in Human Breast Tissues

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ABSTRACT

***Objective*: The absorption spectrum obtained using diffuse reflectance measurements of malignant, fibroadenoma, and normal human breast tissues were studied. The spectral features in the spectrum were assigned to molecular components in the tissues. *Background Data*: Over the past decade, the methods of fluorescence, excitation, and Raman spectroscopy have been studied as potential noninvasive diagnostic tools. Useful spectroscopic information may be obtained from absorption spectra of tissues as well. However, direct measurement of absorption spectra of tissues by conventional transmission means is complicated by multiple photon scattering in tissues. Diffuse reflectance spectrum offers an indirect way to obtain absorption spectrum. *Methods*: Excised malignant, fibroadenoma, and normal breast tissue samples without any treatment were obtained from pathology. Samples were placed in a quartz cuvette. The diffuse reflectance measurements between 250 nm to 650 nm were performed using an automated dual lamp spectrophotometer. The absorption spectra of breast tissues were obtained from the diffuse reflectance measurement. *Results*: Twenty-one invasive carcinoma, 20 mixed in situ and invasive carcinoma, 14 fibroadenoma, and 39 normal breast tissue samples were studied. The absorption spectra of breast tissues were obtained from diffuse reflectance spectra. Spectral features were assigned to DNA and proteins in human breast tissue. Amplitude of changes averaged over 275 nm to 285 nm and 255 nm to 265 nm and were found to be different for malignant, fibroadenoma, and normal breast tissues. These changes arise from differences in content of protein and DNA. *Conclusion*: The peaks of absorption spectrum derived from diffuse reflectance measurements in the UV region revealed fingerprints from proteins and DNA components. The absorbance in the wavelength ranges of 275–285 nm and 255–265 nm were found to be different for malignant, fibroadenoma, and normal breast tissues. These differences provide a criterion to distinguish malignant from fibroadenoma and normal breast tissues.**

INTRODUCTION

There are qualitative and quantitative changes in the nucleic acid and proteins between normal and carcinoma in situ and invasive states. The carcinoma cell in situ is composed of an abundance of special proteins in the cytoplasm and irregular nuclei. In invasive carcinoma, malignant cells invade the stroma, and changes in the extracellular matrix occur. Fluorescence[1,2] and excitation[3,4] optical biopsy spectroscopy from native human tissues have been studied as a potential noninvasive clinical tool for cancer diagnostic purposes. The differences between malignant and normal breast tissue observed in the excitation spectra have been shown to reveal changes of proteins in the cellular and extracellular matrices of malignant breast tissues. The fluorescence and excitation spectrum cannot be used to relate these changes to DNA in tissue because the fluorescence yield of DNA at room temperature is very low (about 4×10^{-5}). In contrast, the fluorescence yield from amino acid (tyrosine and tryptophan) is larger, ~0.20.[6] The absorption spectrum of tissue can be related to the change of protein and DNA in tissue; however, tissue is a highly scattering medium. Due to multiple photon scattering, direct measurement of absorption spectra by conventional transmission methods is not easily done. Dif-

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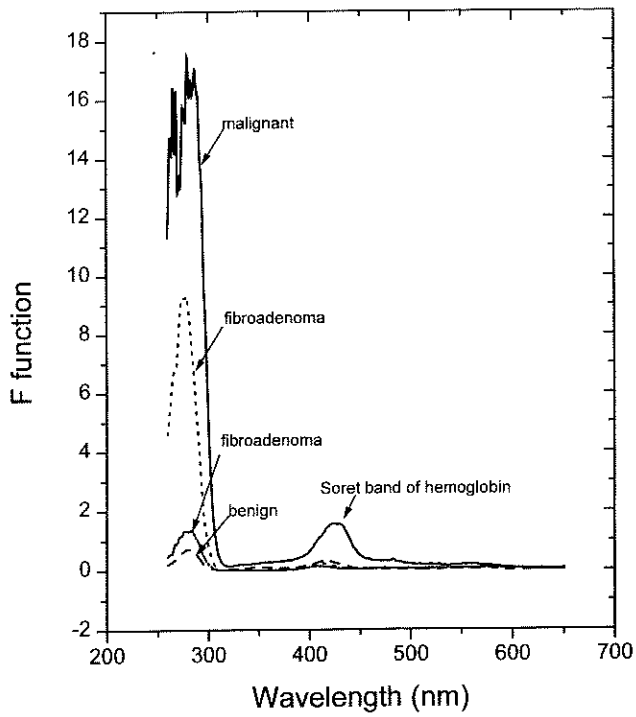

FIG. 1. Transformed absorption spectrum of typical normal, fibroadenoma, and malignant breast tissues.

fuse reflectance spectrum offers a powerful way to obtain a measure of the absorption spectrum[7] in situ.

In this paper, the diffuse reflectance spectrum was used to obtain the transformed absorption spectral function for various type of breast tissues. The peaks of transformed absorption spectrum in the UV region revealed fingerprints from proteins and nucleic acid (DNA) components. The amplitude at wavelengths 275 nm to 285 nm and 255 nm to 265 nm were found to be different for malignant, fibroadenoma, and normal breast tissues. These differences gave rise to a criterion to distinguish between malignant from fibroadenoma and normal breast tissues.

METHODS

Materials

Excised normal, adipose, fibroadenoma, and malignant breast tissue samples were obtained from St. Vincent Hospital, Memorial Sloan Kettering Cancer Center, and the National Disease Research Interchange (NDRI). Specimens were neither chemically treated nor frozen and were stored in the refrigerator before spectroscopic measurements. The interval between sample excision and measurement was less than 24 h. The size of each specimen was about $1 \times 3 \times 0.2$ cm. Samples of random shapes were mounted in a $1 \times 1 \times 5$-cm commercial quartz cuvette and closely attached to its inner surface to obtain a more intense optical signal. Usually, up to three different locations were measured for each specimen. The malignant breast tissue specimens were classified into invasive carcinoma and mixed in situ and invasive carcinoma (part in situ and part invasive), according to the pathology report. Samples studied were 21 invasive carcinoma, 20 mixed in situ and invasive carcinoma, 14 fibroadenoma, and 39 normal breast tissues.

Instrument

The diffuse reflectance spectrum was performed using an automated dual lamp-based spectrophotometer (Mediscience Technology Corp.), based on Perkin-Elmer LS-50 Spectrophotometer. The measurements of diffuse reflectance spectra were selected using a synchronized scan mode, in which the emission and excitation monochromators were scanning on the same wavelengths synchronously.

Model

The transformed absorption spectum is derived from a basic equation for diffuse reflectance from a layer composed of absorbing and light-scattering particles that are uniformly and randomly distributed. The absorption spectral function is given by:

$$F \equiv (1 - R_\infty)^2/2R_\infty, = K/S \qquad (1)$$

The absorption coefficient K is the fraction of the light absorption per unit path length in the sample, and S is the fraction of the light, which is scattered in the unit path length.

The diffuse reflectance signal $R(\lambda)$ depends on the characteristic (absorption and scatter) of the specimen and the dispersion of the instrument. A white scatter standard material with no absorption, ($k = 0$) in the spectral region of interest was used as reference. When this condition is truly fulfilled, $R_{\infty,\mathrm{STD}} = 1$. In our test experiments, the standard scatter was certified Spectralon™ 99% Reflection Standard (Labsphere, North Sutton, NH).

RESULTS

The transformed absorption function values from the reflective spectrum for malignant, fibroadenoma, and normal breast

TABLE 1. THE AVERAGED VALUE OF TRANSFORMED ABSORPTION SPECTRUM $F(r_\infty)$ AT 275 NM–285 NM AND 255 NM–265 NM FOR DIFFERENT KINDS OF BREAST TISSUES

Type of specimens	*Size*	*Averaged at 275 nm to 285 nm*	*Averaged at 255 nm to 265 nm*
Invasive carcinoma	84	13.11 ± 10.39	19.05 ± 18.70
Mixed invasive and in situ	80	5.23 ± 4.16	8.78 ± 13.30
Normal	97	0.88 ± 0.69	0.66 ± 0.70
Fibroadenoma	50	5.23 ± 5.69	3.62 ± 4.41

tissues are displayed in Fig. 1. Each specimen was measured at least at three different locations. The averaged size of different tissue is also given in the Table 1. The peak near 280 nm appears for all the tissues with different amplitudes. The tissue is not homogeneous, but the amplitude of the malignant tissue is still higher than the critical value for fibroadenoma and normal tissues. This peak is associated with absorption by proteins. The salient peak near 265 nm is very prominent and distinct for malignant tissues, and corresponds to DNA. For fibroadenoma, the magnitude of the peak near 280 nm varies and the peak at 265 nm is not as apparent as compared to the malignant tissue. The amplitude at 265 nm is much smaller for normal and fibroadenoma tissues.

Figure 2 shows relative absorption of malignant breast tissue, which was transformed from the diffuse reflectance spectrum. The absorption spectra from DNA and amino acid tryptophan are also displayed in Fig. 2. It is clear that the peaks correspond to the absorption peak of DNA and amino acids. It appears the DNA and protein absorption peaks of the tissue can be obtained from diffuse reflectance (DR).

DISCUSSION

Fibroadenoma is usually found in younger women, and is the most common neoplasm of the breast. This tumor is composed of epithelial and stromal cells that originate from the terminal duct lobular unit. It is important to distinguish fibroadenoma from malignant tumors. The averaged value of the absorption function $F(r_\infty)_{275\text{nm}-285\text{nm}}$ for fibroadenoma was 5.23 ± 5.69, which is a higher value than 0.88 ± 0.69 for normal tissue. The peak of protein cannot distinguish fibroadenoma from malignant tissue. From Table 1, it was found that the averaged amplitude of $F(r_\infty)_{255\text{nm}-265\text{nm}}$ (19.05 ± 18.70) is higher than $F(r_\infty)_{275\text{nm}-285\text{nm}}$ (13.11 ± 10.39) for malignant tissue. For fibroadenoma tissue, the averaged amplitude of $F(r_\infty)_{255\text{nm}-265\text{nm}}$ (3.62 ± 4.41) is lower than $F(r_\infty)_{275\text{nm}-285\text{nm}}$ (5.23 ± 5.69). This finding is especially critical because it is important to distinguish fibroadenoma from malignancies promptly.

The experimental data from different kind of tissues was plotted in Fig. 3 with log $[(F)_{275\text{nm}-285\text{nm}}]$ as the x axis and log $[(F)_{255\text{nm}-265\text{nm}}]$ as the y axis. The location of each specimen is

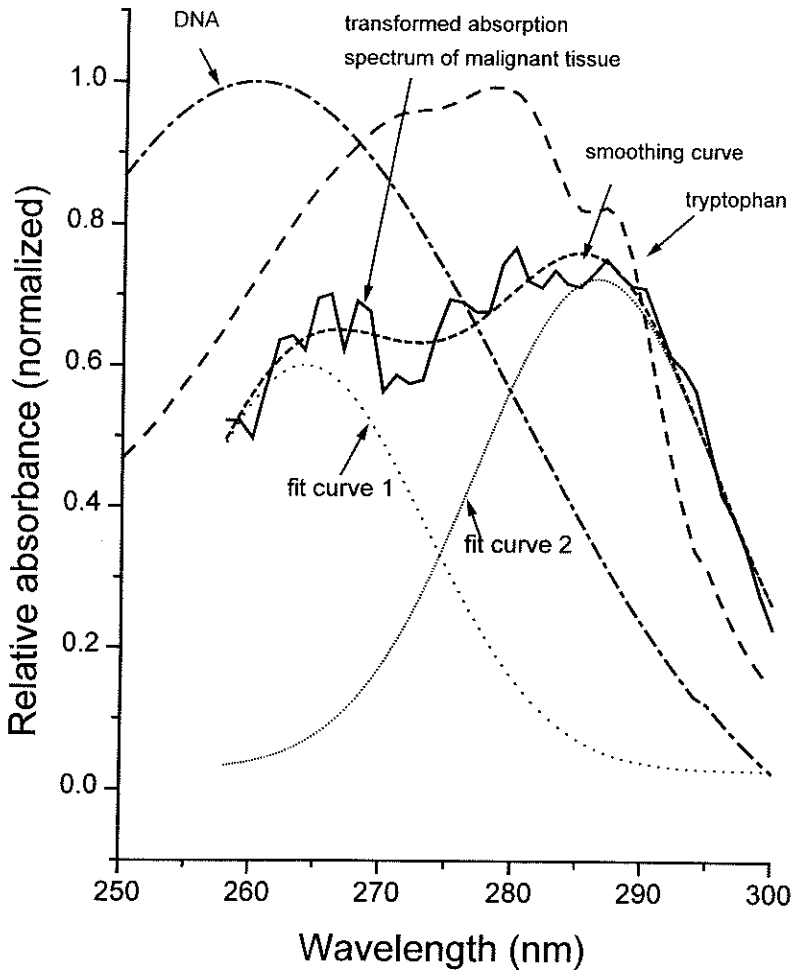

FIG. 2. Transformed absorption spectrum of malignant breast tissue and the absorption spectrum of DNA and the amino acid tryptophan. Two fit curves for malignant breast tissue were also provided.

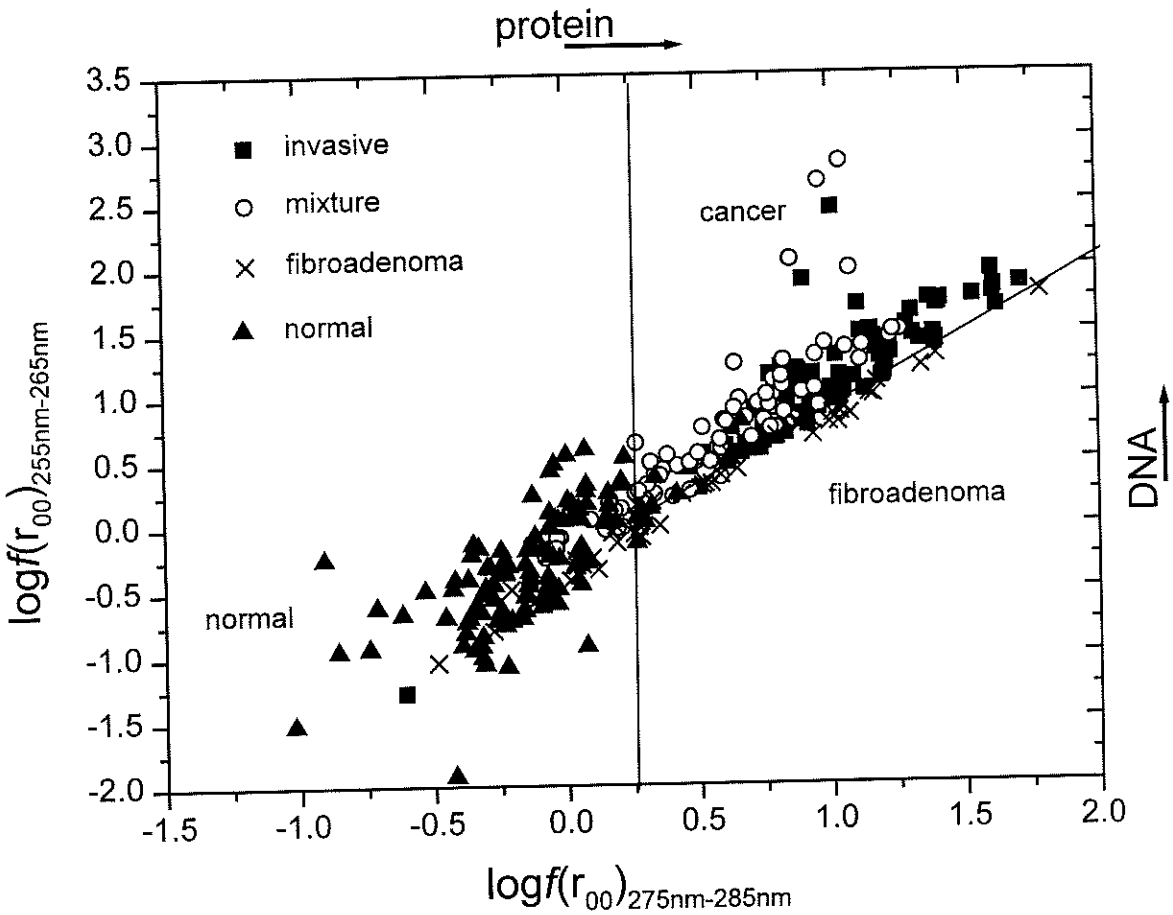

FIG. 3. Scatter data plot of invasive carcinoma, mixed of invasive and carcinoma in situ, fibroadenoma, and normal tissues of log $[(F)_{275nm-285nm}]$ as x axis and log$[(F)_{255nm-265nm}]$ as y axis.

related to the absorption of DNA and proteins. From the data in Fig. 3, one can find that different kinds of specimens have their own zone in this special coordinate system. For normal tissues, the absorption of proteins is lower with less DNA absorption. For malignant tissues, the proteins' absorption is higher, with larger DNA absorption also apparent. For fibroadenoma tissue, sometimes even the absorption of proteins was as high as malignant tissue, but the DNA absorption is smaller. Together with the change of absorption of DNA and proteins, malignant tissue can be separated not only from normal tissue but also from fibroadenoma. This observation is expected because in pathology DNA contents is found to increase when tissue changes to the malignant state.

CONCLUSION

The transformed absorption spectrum in the UV spectral range was used to determine the peaks at 265 nm and 285 nm due to DNA and proteins, respectively. For malignant tissue, the DNA peak was prominent whereas for normal tissues the absorption of proteins was lower and there was smaller DNA absorption. Furthermore, for fibroadenoma tissue, although protein absorption was sometimes in the range of malignant samples, smaller DNA absorption was found. The absorption spectral function gives information about DNA and proteins content in tissues.

ACKNOWLEDGMENTS

We thank Dr. N. N. Zhadin, Dr. S. Gayen, and Mr. Wei Cai, Mr. Gang Zhang for helpful discussions. The work was supported in part by Organized Research of CUNY, and the Department of Energy (DOE).

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