Tryptophan Fluorescence of Cells and Tissue in Esophageal Carcinoma

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***Abstract*— Cancer is a cellular process. The emission spectrum of tryptophan, which produces the strongest fluorescence in cells, was investigated in cells and tissues of a normal and malignant esophagus. Estimated fluorescence intensity per cell was about three times greater in cancerous cells than in normal cells. The fluorescence was also greater in cancerous tissue but the difference was attenuated, probably because of absorption and scattering. Cellular fluorescence from tryptophan may be useful for the detection of cancer in esophageal cells and tissues.**

***Index Terms*— Autofluorescence, cancer, tryptophan.**

I. Introduction

CANCER of the esophagus is a disease with a very poor prognosis where a favorable outcome is limited to patients with very early, often pre-symptomatic flat lesions found on random biopsies. A number of optical detection methods have been used to facilitate the detection of early cancer that is invisible to the naked eye, including reflectance, light scattering and autofluorescence [1]. Autofluorescence has the advantage of being able to accomplish real time wide-field macroscopic imaging without requiring exogenous agents. A band of emission from 400 to 650 nm, with contributions from cellular NADH and extracellular collagen has been used for endoscopic imaging, where an overall decrease in fluorescence intensity is seen in cancer compared to normal tissue; however, this signal was not shown to be superior to random, blind biopsies and produced a high rate of false positive images in the presence of inflammation [2]. Native tissue fluorescence has contributions from molecules within cells (tryptophan, tyrosine, NADH and FAD) as well as the extra-cellular matrix (such as collagen and elastin). The emitted fluorescence is also scattered and absorbed. Since cancer is, a priori, a cellular process where cancerous cells multiply in an uncontrolled manner, we studied the fluorescence of the essential amino acid tryptophan in dissociated cells of the esophagus as well as in esophageal tissue. Tryptophan is the predominant source of cellular fluorescence in the UV [3] and a recent publication supports the feasibility of imaging at mid-UV wavelengths [4].

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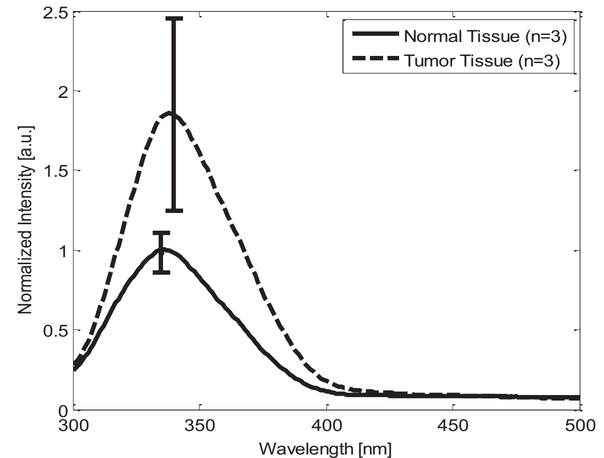

Fig. 1. Emission spectra of the normalized mean emission intensity in arbitrary units of the normal mucosa and adenocarcinoma of the esophagus in three patients. Fluorescence intensity is plotted against wavelength, which shows greater peak emission intensity for cancerous tissue at 330–340 nm ± SD.

II. Materials and Methods

Surgical specimens of the normal and cancerous distal esophagus from three patients (mean age 67) with confirmed esophageal adenocarcinoma were immediately snap frozen in liquid Nitrogen and stored at −70 degrees Celsius.

Specimens were later thawed over ice and a scalpel was used to obtain samples of tumor and normal mucosa, about 5 mm square and 1 mm thick. Each sample was individually mounted on a specimen holder with a matte black surface inside the spectrofluorometer (Shimatzu RF- 5301 PC Columbia, MD) with the mucosal side facing the excitation beam. A 150 Watt Xenon lamp provided the excitation beam, with an accuracy of ± 1.5 nm, a slit width of 1.5 mm and a wavelength range of 220 nm to 900 nm. Excitation intensity varied with wavelength but was always less than 5 μW/mm^2. Each specimen was excited at 290 nm and emission intensity measured from 300 nm to 500 nm at 1 nm increments. All studied specimens were then submitted for histology.

Further samples of tumor and normal mucosa (approximately 1 gram each) were obtained from a single patient's specimen with a scalpel. Using an established technique, cells were separated from the extracellular matrix to produce a suspension of cells in a solution of phosphate buffered saline (PBS) at pH 7.4 [5]. The cell samples were placed in a quartz cuvette in the same spectrofluorometer and the emission spectra recorded as with tissue samples. Using a hematocytometer (Fisher Scientific, Pittsburgh, PA) the concentration of cells in each sample was recorded and their viability confirmed with trypan blue stain aversion.

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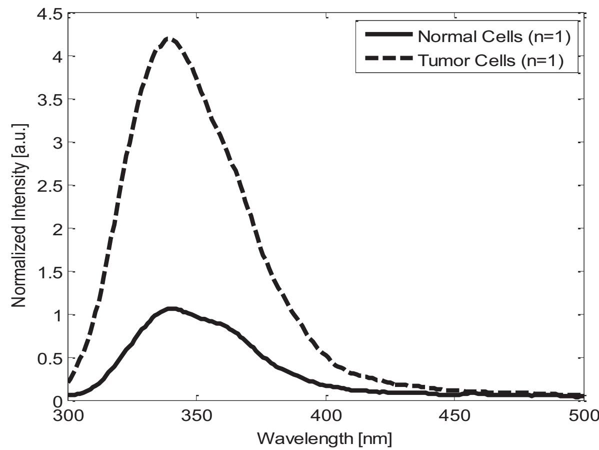

Fig. 2. Emission spectra of normal and cancerous cells of the esophagus in PBS at pH 7.4 with fluorescence intensity in arbitrary units plotted against emission wavelength, which show much greater peak emission intensity in cancerous cells compared to normal cells.

TABLE I
ESTIMATED INTENSITY OF FLUORESCENCE PER CELL

	Cancer cells	Normal cells
Estimated number of cells/μl	1000	750
Peak fluorescence intensity in arbitrary units	14.7	3.7
Peak fluorescence intensity (arbitrary units)/cell/μl	0.0147	0.0049

III. RESULTS

Mean normalized emission spectra of normal esophageal mucosa and esophageal cancer are shown in figure 1. Figure 2 shows normalized emission spectra of normal and cancerous cells in PBS from a single patient. A prominent emission peak was seen at 330 nm to 340 nm in both tissues and cell suspensions. The maximum mean emission intensity of cancerous tissue was almost twice that of normal esophageal mucosa. The maximum intensity of the cancerous cell suspension was about four times greater than that of normal cells (figure 2). Trypan blue staining showed about 80% of the cells in both samples to be viable. To correct for the concentration of cells, the peak intensity of fluorescence in each cell suspension was divided by the concentration of cells in that sample, to estimate the intensity of fluorescence per cell/μl. The results are shown in table 1.

IV. CONCLUSION

This is the first description of tryptophan fluorescence in tissue samples and extracted cells of the esophagus and shows greater fluorescence in cancerous tissue and cells, compared to normal. Fluorescence spectra of tissues and cells with an emission peak at 330–340 nm, with excitation at 290 nm is consistent with the emission from tryptophan [6] and has been described in human tissues [3]. Tryptophan is an essential amino acid is a component of proteins and cells. Cancerous cells undergo accelerated and uncontrolled mitosis, contain a larger cell nucleus and have a greater nuclear protein content than normal cells; the increased intensity of this cellular fluorescence correlates with this pathological process [7].

Fluorescence, attributed to tryptophan was about three times greater in cancerous cells than in normal cells of the esophagus (see table 1), whereas fluorescence intensity of cancerous tissue was approximately twice that in normal mucosa (figure 1). This attenuation of net tryptophan fluorescence in esophageal tissue compared to isolated cells may be due to scattering and absorption of fluorescence by cells, connective tissue and blood vessels. Absorption of fluorescence by hemoglobin would also be greater in cancerous tissue due to the increased vascularity seen in malignancy [8].

Tryptophan related cellular fluorescence may be used to detect cancerous cells and flat, early esophageal cancer. UV transmitting illumination and collection optics can facilitate endoscopic readouts. Any spectroscopic or imaging method that utilizes this signal in tissues will need to make adjustments for the effects of local absorption and scattering and demonstrate the safety of using short wavelengths in vivo.

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