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Protoporphyrin IX fluorescence from the plasma of tumor-implanted mouse

J.W. Meng[a], X.J. Wang[b,*], H.P. Ma[c], X.G. Ren[a], X.R. Xu[a], W.M. Ren[c]

[a]*Changchun Institute of Physics, Academia Sinica, Changchun 130021, People's Republic of China*
[b]*Department of Physics, Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA 30460-8031, USA*
[c]*The People's Hospital of Jilin Province, Changchun 130021, People's Republic of China*

Abstract

MH134 mouse tumor cells were implanted in C3H/HeN mice and the metabolic level of protoporphyrin IX from the tumor-implanted mice was studied. The fluorescence intensity from the plasma of tumor-implanted mice was obtained during the tumor development and the spectral ratio was used to determine the stage. The experimental data of metabolic level was analyzed and fitted to a function $L(t) = L_0 + L_{max}[1 - ((t - T/2)/(T/2))^2]$, which described the tumor development and may be used to estimate the stage of disease in the tumor-implanted mice.

Keywords: Protoporphyrin IX; Tumor; Fluorescence

1. Introduction

When disease occurs in humans or animals, it will affect the metabolism and the regulation of organic matter inside the body. By investigating the metabolic process, the disease can be diagnosed. Abnormal protoporphyrin IX (PpIX) metabolism has been found in cancerous patients by measuring the fluorescence spectra of the cancerous tissue, blood, plasma, and the serum [1,2]. PpIX is an intermediate in the biosynthesis of heme, and can accumulate in cells since the conversion into heme is slow. Although the factors which control the biosynthesis and accumulation of PpIX have not been fully understood, in many situations both cancer cells in vitro and tumor tissue in vivo do accumulate substantially more PpIX than normal cells and tissues [3]. The spectral ratio of the characteristic visible emission spectra of PpIX has been used to monitor change in the metabolism. This fluorometric method for tumor diagnosis has been applied in clinical trials [4]. In addition, 5-aminolevulinic acid-induced PpIX fluorescence has been used for the detection of early bladder cancer [5] and for photodynamic therapy of skin cancer [6]. Emission or excitation spectra in the UV range from some proteins were also used to detect breast cancer [7] and to differentiate normal and malignant tissue [8].

The spectral ratio can be used to monitor the level of PpIX metabolism of a cancerous person or animal since the intensity is linearly dependent on the concentration of the luminous matter. In this

* Corresponding author. Tel.: +1-912-681-5503; fax: +1-912-681-0471.
E-mail address: xwang@gasou.edu (X. Wang)

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paper, the fluorescence intensity of the blood from the tumor-implanted mice was measured and the development of PpIX metabolism from cancerous patients studied. Experimental data of PpIX metabolism was fit to the function that may be used to establish a fluorometric method for estimating the stage of the tumor development.

2. Sample preparation and experimental method

For animal experiments, a suspension of MH134 mouse tumor cells was prepared. MH134 mouse tumor cells were implanted in the C3H/HeN mouse peritoneal cavity to propagate the cells. The tumor ascites was aspirated and washed twice using saline. The inactive suspension of tumor cells was made to a concentration of 8.8×10^7 cells/ml.

MH134 mouse tumor cells were inoculated in 30 female C3H/HeN mice (weight: 19.0 ± 0.9 g; age: 9 weeks). Each mouse was injected with 0.1 ml of the inactive suspension of tumor cells in its subcutaneous tissue of the right hind legs. The tumor cells grew in the back of the tumor-implanted mice and could be observed after 5 days.

The 30 tumor-implanted mice were divided into 10 groups of three each. Three normal mice formed another group as control. Starting the control group, 11 blood samples were collected from different groups for every three days. Following sodium pentobarbital anesthesia, the blood was obtained from the heart by opening the thoracic cavity. The blood samples were centrifuged ($2000 \times$ g) for 10 min at 4°C to remove corpuscles and then the plasma was obtained. Heparin (0.1%) was used as an anticoagulant. Both blood and plasma samples were preserved in a darkroom.

Blood samples from human patients with liver cancer were freshly drawn from a elbow vein in the early morning (before eating any food, 3 ml each patient). Alcohol and medicine were forbidden to be used by the patients for 3 days prior the draw.

Fluorescence spectra were measured using Hitachi F-4000 spectrofluorometer with excitation wavelength at 390 nm.

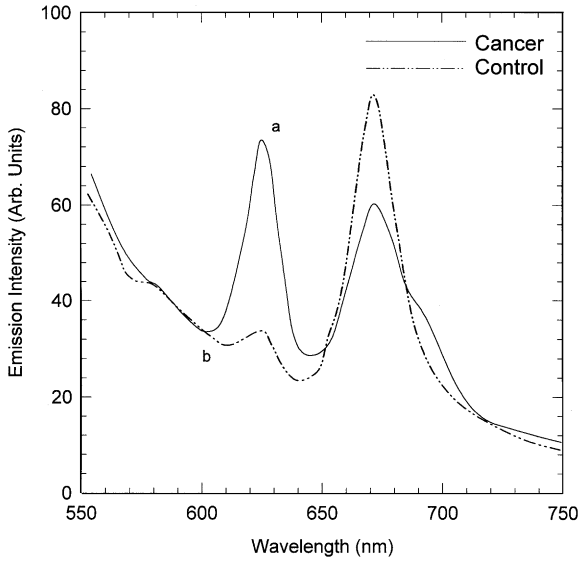

Fig. 1. Emission spectra from PpIX. Solid line represents the emission from sample of the tumor-implanted mouse, while the dashed line represents that from a normal mouse.

3. Results and discussion

Fig. 1 shows a typical fluorescence spectrum from the plasma sample. The solid line and dashed line represent the emissions from the tumor-implanted mouse and the normal mouse, respectively. Strong fluorescence emission around 620–630 nm was observed from the plasma of the tumor-implanted mouse. The characteristic emission and its background are due to the PpIX and proteins, respectively, indicating that the level of PpIX metabolism evidently arises for the tumor-implanted mouse. If L represents the level of PpIX metabolism, it may be defined by

$$L = \frac{I_a - I_b}{I_b}, \tag{1}$$

where I_a is the peak fluorescence intensity of PpIX at point a; I_b is the fluorescence intensity of background at point b. As shown in Fig. 1, $L \approx 0.1$ for normal mouse and $L \approx 1.3$ for tumor-implanted mouse, respectively.

The process of PpIX metabolism in tumor-implanted mice was studied by measuring the fluores-

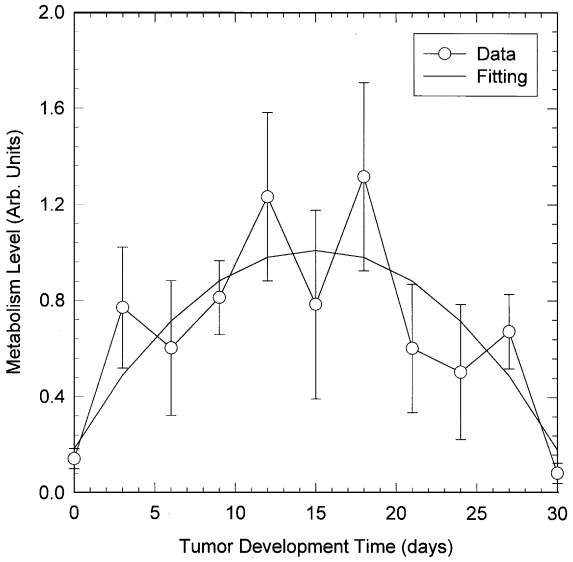

Fig. 2. PpIX Metabolism level versus the tumor development time. The line connecting the data points is added as a visual aid. The smooth solid line is fitted to the data by the function given by Eq. (2).

cence intensity of the plasma from the mice. Fig. 2 shows the PpIX metabolism level, L, against the development time after tumor implantation. The dots represent the average value of L for each group. The line connecting the data points is added as a visual aid. The solid line is fitted to the data by a function that will be discussed in the next section. At day 3, as shown in Fig. 2, although there is no pathological change found in the back of the tumor-implanted mice, the L value from the plasma of the mice is higher than that from the normal mice. This suggests that PpIX metabolism is affected by the early stage tumor development. This may provide an early diagnosis for cancer. The level of the PpIX metabolism increases with the time of the tumor implantation and reaches its maximum value around day 15 when PpIX was most abundant. Thereafter, the metabolic level of PpIX will decrease as the nutrition and energy stores are exhausted.

The following equation was used to fit the data of the PpIX metabolism level as a function of the tumor development time (t) in Fig. 2.

$$L(t) = L_0 + L_{\max}\left[1 - \left(\frac{t - T/2}{T/2}\right)^2\right] \quad (0 \leqslant t \leqslant T), \tag{2}$$

where L_0 is the initial value of L, i.e., the PpIX metabolism level of the normal mouse. T is the life period of the tumor-implanted mouse from implantation to death. $T/2$ is the half of the period corresponding to the maximum PpIX metabolism level of the plasma from the tumor-implanted mouse, $L_{\max}$. L_0 is dependent upon the race of animal and its food and drinking habits, of while $L_{\max}$ is related to the type of tumor.

Eq. (2) can be used to estimate the stage, t, of the disease. There are two stages corresponding to a given $L(t)$. To determine if the stage is before or after the half-period, the tumor's volume, V, which is monotonically increasing with time, will be used. V needs to be measured and compared to $V_{\max}$, the umor's volume at the half-period. $V_{\max}$ can be statistically obtained from experimental results. When $V < V_{\max}$, the tumor is at an early stage, and when $V > V_{\max}$, the tumor is at a late stage. For C3H/HeN mice: $V_{\max} = 1676\,\mathrm{mm}^3$ [4], $T = 30$ days, and $L_{\max} = 0.7$. For example, when L is measured at a value of 0.6 and V at a value of $878\,\mathrm{mm}^3$ that is smaller than $V_{\max}$, the tumor is at an early stage and $t = 9$ days. If, for the same L, V is greater than $V_{\max}$, $t = 21$ days and the tumor is at a late stage.

Fig. 3 shows two typical emission spectra of the plasma samples from the normal patients (dashed line) and the patients with liver cancer (solid line), respectively. Emission around 625 nm originates from the PpIX. Similarly to the mouse experiments, the PpIX content in cancerous patients' blood was clearly higher than that in the normal patients.

In summary, the metabolism level of PpIX of the tumor-implanted mice was studied using fluorometry. The fluorescence intensity of plasma from the tumor-implanted mice was measured during the tumor development. The experimental data was analyzed and fitted to a function $L(t) = L_0 + L_{\max}[1 - ((t - T/2)/(T/2))^2]$, which may be used to estimate the stage of the disease of the tumor-implanted mice. Stronger PpIX emission in patients with liver cancer was also observed.

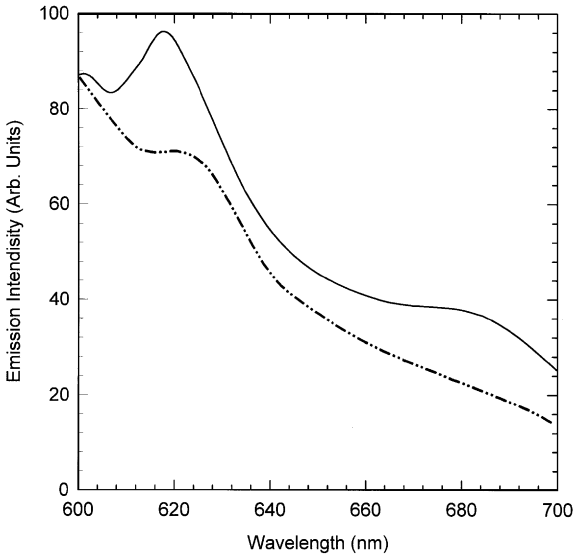

Fig. 3. Typical emission spectra of the plasma sample from the normal patients (dashed line) and the patients with liver cancer (solid line).

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