

Study of Blood Porphyrin Spectral Profile for Diagnosis of Tumor Progression

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Received: 5 December 2006 / Accepted: 14 February 2007 / Published online: 29 March 2007
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Abstract Renal cell carcinoma (RCC) accounts for approximately 3% of new cancer incidence and mortality in the United States. Unfortunately many RCC masses remain asymptomatic and nonpalpable until they are advanced. Diagnosis and localization of early carcinoma play an important role in the prevention and curative treatment of RCC. The autofluorescence of blood porphyrin of healthy and tumor induced in male SCID mice was analyzed using fluorescence and excitation spectroscopy. A significant contrast between normal and tumor blood could be established. Blood porphyrin fluorophore showed enhanced fluorescence band (around 630 nm) in function of the tumor growth. This indi-

cates that either the autofluorescence intensity of the blood fluorescence may provide a good parameter for the “first approximation” characterization of the tumor stage.

Keywords Tumor · Porphyrin · Fluorescence · Tumor growth

Introduction

Renal cell cancer (RCC) accounts for ~3% of human malignancies and its incidence appears to be rising. RCC is frequently asymptomatic and, for this reason, at the moment of the diagnosis, it was advanced and many times, the metastasis was established [1]. Diagnosis and localization of early carcinoma play an important role in the prevention and curative treatment of colonic cancer. However, physical biopsies do not fully solve this problem because they are sampled at random locations, which is highly dependent on the skill and experience of the investigator. As a result, a significant number of lesions, especially the carcinoma in situ lesions, are not sampled and subsequently diagnosed [1].

Fluorescence technique appears very important for the diagnosis of cancer. Fluorescence detection has advantages over other light-based investigation methods: high sensitivity, high speed, and safety.

Protoporphyrin IX is a porphyrin derivative that combines with ferrous iron to form the heme of hemoglobin and with ferric or ferrous iron to form the prosthetic groups of substances such as myoglobin, catalase, and the cytochromes. Abnormal metabolism of Protoporphyrin IX has been observed in the blood, plasma, serum and tissue of cancerous patients, which indicates that cancer cells accumulate substantially more Protoporphyrin IX than the normal cells and

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tissues [2–5]. Protoporphyrin IX is also the predominant porphyrin in the blood [6].

In this work we used autofluorescence spectroscopy of porphyrins which can be extracted from blood by acetone [7] to distinguish between normal and cancer specimens. Red fluorescence from blood porphyrins of tumor induced in male SCID mice was observed experimentally by examination with ultraviolet (UV) irradiation. The objective of the current study was to examine correlation of red fluorescence and tumor growth to search for a possible method for estimating the stages of the tumor development.

Material and methods

Cell line and cell culture conditions

Caki-1 cells were purchase from ATCC were cultured in DMEM containing high glucose (4.5 g/l at 25 mM) and supplemented with 100 units/ml penicillin, 50 mg/ml streptomycin, and 10% FBS. The cells were maintained in a humid chamber at 37°C in an atmosphere of 5% CO₂.

Animals and tumor induction

A total of eighteen male SCID mice, ~6 weeks old on arrival, were obtained from the CEDEME UNIFESP-EPM and housed in laminar airflow cabinets under pathogen-free conditions with a 12-h light/12-h dark schedule and fed autoclaved standard chow and water *ad libitum*. Mice were injected sc in the left hind flank with 4.6×10^6 cells/mouse in a volume of 0.1 ml of sterile phosphate buffered saline (PBS).

Monitoring of tumor growth

The animals were monitored for the presence of palpable tumor. After identification of tumor mass, tumor dimensions were measured and blood samples were collected at 7, 13, 20 and 28 days. Approximately 0.1 ml of blood was collected with EDTA as anticoagulant by retro-orbital plexus with glass capillary for each animal. Tumor dimensions were measured with electronic calipers and volumes were calculated as follow: Tumor volume = length \times width² \times 0.52. All experiments were performed in accordance with institutional guidelines for animal care.

Porphyrin extraction

Collected blood was centrifuged at 4000 rpm for 15 min. The supernatant plasma was removed completely and two volumes of analytical grade acetone was added in the formed elements and mixed well. The mixture was centrifuged by

using the same conditions described above. The clear supernatant of mixture was collect in a clean tube and maintained at 4°C before spectrofluorometer analysis.

Fluorescent spectral analyses

The emission spectra were obtained by exciting the samples, inside a 1 mm optical path cuvette, with a 150 W Xenon lamp. The emissions of the samples were analyzed with a 0.5 m Spex monochromator and a S-20 PMT detector. The signal was amplified with an EG&G 7220 lock-in and processed by a computer.

Results

It is known that in the UV-visible absorption spectrum, the porphyrin shows intense absorption at around 417 nm (the “Soret” band), followed by several weaker absorptions (Q Bands) at higher wavelengths (450 to 700 nm) [8]. Variations of the peripheral substituents on the porphyrin ring often because minor changes to the intensity and wavelength of these absorptions. Protonation of two of the inner nitrogen atoms or insertion of a metal into the porphyrin cavity also changes the visible absorption spectrum. These absorptions can often be very helpful in determining certain features on a porphyrin. Considering this fact the blood samples of both normal and tumor specimens were excited at 420 nm and the emission spectra showed in the Fig. 1 was obtained. By this spectrum we observe two emission bands centered around 632 and 699 nm. These peaks in the red are characteristic peaks of Protoporphyrin IX. We observe a high important difference in the intensity of emission bands when we compare normal and tumor blood sample. The emission band at 632 nm is more intense in the tumor blood in comparison

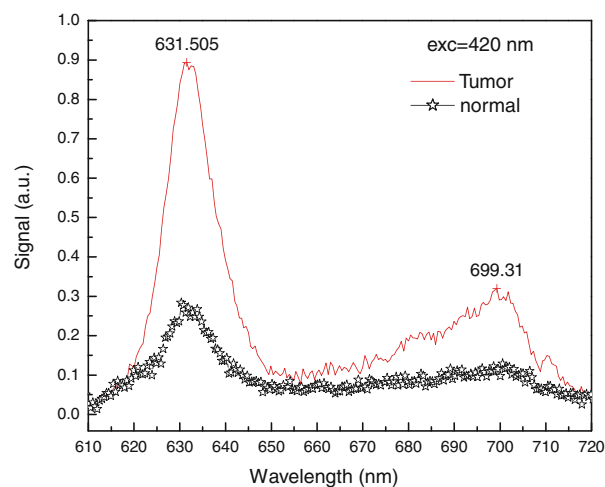


Fig. 1 Emission spectra of porphyrins of normal and tumor blood sample obtained fixing excitation at 420 nm

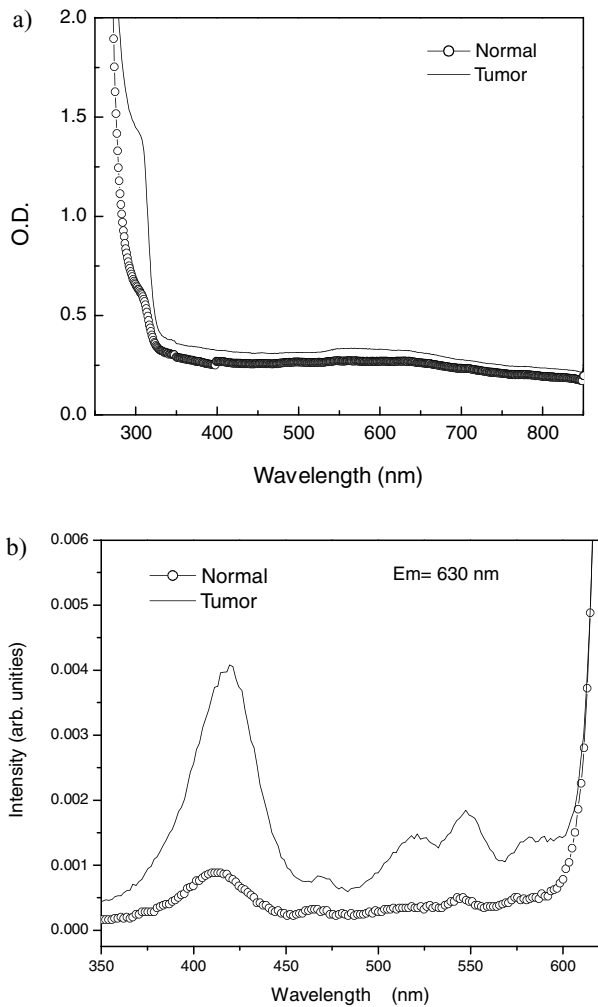


Fig. 2 (a) Absorption spectra for normal and tumor blood sample and (b) excitation spectra of blood porphyrin obtained fixing emission at 630 nm

with normal blood. The observed effect can indicate that cancer blood have substantially more Protoporphyrin IX than the normal blood.

In the Fig. 2b we shown the excitation spectra obtained for normal and tumor induced blood samples showing differences in the intensities of both Soret and Q bands obtained fixing emission at 630 nm. In this case different intensities for the excitation bands for normal and tumor samples can also be observed. In the absorption spectra observed in the Fig. 2a for the same samples is not possible to distinguish the Soret and Q bands but differences in the optical density around 300 and 580 nm for normal and tumor can be observed. Considering the differences in these spectra we believe that is possible to obtain correlation between absorbance and cancer growth with absorption measurements. However, contrary to the absorption spectra, the emission spectra is related only to the porphyrin signal without con-

Table 1 Tumor progression

Cam	Tumor volume (mm ³)			
	7 days	13 days	20 days	28 days
1	91	151.2	151.2	326.6
2	*	Died		
3	*	31.59	47.4	44.8
4	Died			
5	34	*	*	*
6	10.05	Died		
7	Died			
8	66.27	136.21	213.3	608.19
9	61.1	78.17	293.52	577.72
10	*	*	185.32	died
11	73.34	168.35	175.82	584.9
12	72.82	71.98	67.62	
13	64.21	168.31	Died	
14	76.98	158.7	189.7	504.8
15	*	150.1	222.42	108.12

tribution of other blood constituents or acetone and for this reason we consider more appropriated for a study of cancer growth.

Table 1 shows the tumor volume of studied animals in function of days. For these data we obtained the graphic plotted in the Fig. 3.

The fluorescence of blood samples were studied in the first thirteen days after tumor induction and results are shown in the Table 2 and the Fig. 4. All measurements were obtained with the same experimental setup: slits of excitation (input and output ~1 mm) and emission monochromators (input ~500 μm and output ~300 μm), excitation band fixed at 420 nm, same geometry and same optical paths were maintained. To obtain the graphic shown in Fig. 4, the fluorescence intensity at 630 nm for each studied sample are compared. We observe a growth of fluorescence intensity at 630 nm

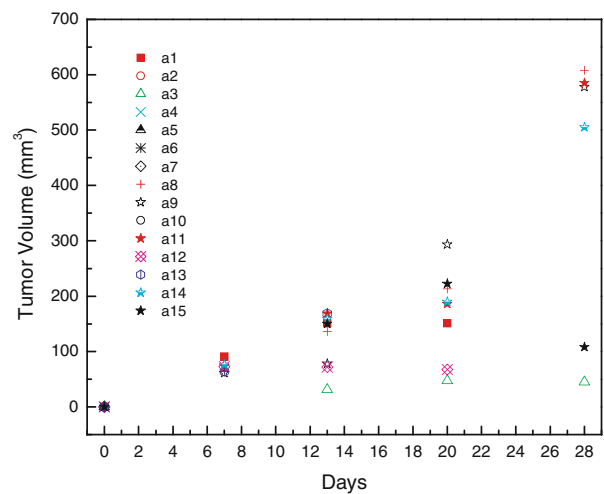


Fig. 3 Tumor growth for normal, 7, 13, 20 and 28 days after tumor induction

Table 2 Fluorescence intensity for normal and 5, 7 and 13 days after tumor induction and statistics on rows

	A1	A3	A5	A8	A9	A11	A12	A13	A4	A15	A16	A17	A18
Normal	–	–	–	–	–	–	–	–	–	–	0.0045	0.0065	0.0088
Palpable	–	0.0069	–	0.0241	0.0265	–	0.0372	–	0.0185	–	–	–	–
7 days	–	–	0.1006	0.0462	0.0302	0.0322	0.0868	0.0835	0.0284	–	–	–	–
13 days	0.142	–	–	0.1007	0.1131	0.0447	0.0877	–	–	0.0654	–	–	–
	Mean	SD (year ±)	SE (year ±)	Min (y)	Max (y)	Range							
	(Y)					(y)	N						
Normal	0.0066	0.00214	0.00124	0.00449	0.00877	0.00428	3						
Palpable	0.02265	0.01109	0.00496	0.00692	0.03717	0.03025	5						
7 days	0.05825	0.03096	0.01117	0.0284	0.1006	0.0722	7						
13 days	0.0922	0.0345	0.01409	0.04471	0.1417	0.09699	6						

in function of days. Through the experimental results it can be observed that the average values of the emission spectra areas of the samples grow in function of the tumor growth indicating accumulation of porphyrin in the blood.

Elucidating the basic mechanisms of tumor growth is one of the most intricate problems in the field of tumor biology, and one of its major challenges. Many attempts have been made in recent decades to obtain a mathematical model that would allow us to discern these basic features of cell and tumor growth [9, 10]. The obtained results show that is possible to study tumor growth using fluorescence spectroscopy of porphyrin extracted in blood samples. This method is important to be a minimally invasive technique.

Conclusions

In conclusion, the fluorescence and excitation techniques appear quite useful for the diagnosis of tumor progression.

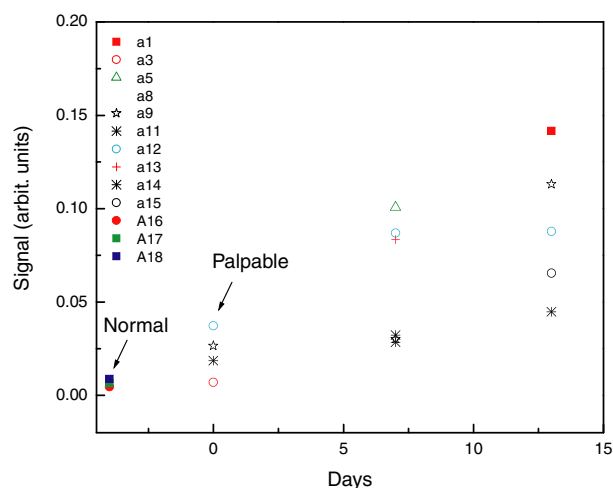


Fig. 4 Fluorescence intensity of blood samples for normal and palpable, 7 and 13 days after tumor induction

Our method has given good results to distinguish between normal and different stages of tumor growth.

The above study shows a reasonable and sizable proof to use this technique for the diagnosis of cancer at different stages and thus, it is a promising technique for mass screening at the primary clinics. Large and well-designed studies are under way to elaborate more on this matter.

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