J Fluoresc (2010) 20:1159–1165
DOI 10.1007/s10895-010-0662-9

ORIGINAL PAPER

Intrinsic Fluorescence of Protoporphyrin IX from Blood Samples Can Yield Information on the Growth of Prostate Tumours

Flávia Rodrigues de Oliveira Silva · Maria Helena Bellini · Vivian Regina Tristão · Nestor Schor · Nilson Dias Vieira Jr. · Lilia Coronato Courrol

Received: 12 November 2009 / Accepted: 30 March 2010 / Published online: 24 April 2010
© Springer Science+Business Media, LLC 2010

Abstract Prostate cancer is one of the most common types of cancer in men, and unfortunately many prostate tumours remain asymptomatic until they reach advanced stages. Diagnosis is typically performed through Prostate-Specific Antigen (PSA) quantification, Digital Rectal Examination (DRE) and Transrectal Ultrasonography (TU). The antigen (PSA) is secreted by all prostatic epithelial cells and not exclusively by cancerous ones, so its concentration also increases in the presence of other prostatic diseases. DRE and TU are not reliable for early detection, when histological analysis of prostate tissue obtained from a biopsy is necessary. In this context, fluorescence techniques are very important for the diagnosis of cancer. In this paper we explore the potential of using endogenous phorphyrin blood fluorescence as tumour marker for prostate cancer. Substances such as porphyrin derivatives accumulate substantially more in tumours than in normal tissues; thus, measuring blood porphyrin concentration by autofluorescence intensity may provide a good parameter for determining tumour stage. In this study, the autofluorescence of blood porphyrin was analyzed using fluorescence and excitation spectroscopy on healthy male NUDE mice and in those with prostate cancer induced by inoculation of DU145 cells. A significant contrast between the blood of normal and cancer subjects could be established. Blood porphyrin fluorophore showed an enhancement on the fluorescence band around 632 nm following tumour growth. Fluorescence detection has advantages over other light-based investigation methods: high sensitivity, high speed and safety. However it does carry the drawback of low specificity of detection. The extraction of blood porphyrin using acetone can solve this problem, since optical excitation of further molecular species can be excluded, and light scattering from blood samples is negligible.

Keywords Porphyrin · Tumour stage · Prostate cancer · Fluorescence · Spectroscopy

F. R. de Oliveira Silva · M. H. Bellini · V. R. Tristão · N. Schor · L. C. Courrol
Departamento de Nefrologia, UNIFESP,
São Paulo, SP, Brazil

N. D. Vieira Jr.
Centro de Lasers e Aplicações, IPEN/CNEN-SP,
São Paulo, SP, Brazil

L. C. Courrol (✉)
UNIFESP,
Campus Diadema,
Diadema, SP, Brazil
e-mail: lcourrol@gmail.com

Introduction

Prostate cancer is the most frequently diagnosed neoplasm in men; it can present as a localized disease within the prostate or may become highly invasive and metastasize to regional lymph nodes and bone. When prostate tumour cells metastasize beyond the confines of the gland, the associated morbidity and mortality of prostate cancer are significant. Curative therapeutic options are limited to localized disease detected early, which is initially done through digital rectal examination and measurement of serum prostate-specific antigen (PSA) [1, 2]. PSA is produced and secreted by glandular cells in the epithelial layer, and the quantity present in the prostate gland increases with the occurrence of prostatic disease. Although regarded as the best conventional serum tumour marker currently available, PSA measurement is not specific

enough for a definite diagnosis of prostate cancer to be established because it also increases in the case of benign growth of the prostate (BPH) or prostate inflammation [3]. The diagnosis of prostate cancer has a high rate false-positive results because of normal cell growth in the prostate. BPH can be histologically observed in approximately 20% of men aged 40 years and older, and this percentage increases to 70% in men aged 60 and older and to as much as 90% in men aged 70 and older. Only 25% of them develop clinical symptoms.

A commonly used method to classify the stage of prostate cancer is the Gleason grade [4], which is based on the patterns of prostatic glands. Lower grades indicate more differentiated carcinomas and therefore less severe tumours. Another method used for diagnosing prostate cancer is Transrectal Ultrasonography [2]. Nevertheless, histological analysis of prostate tissue is necessary for early detection. These traditional techniques rely on tissue being removed and then examined away from the patient.

Fluorescence techniques are very important for the diagnosis of cancer. Fluorescence detection has advantages over other light-based investigation methods: high sensitivity, high speed, and safety [5, 6]. Non-invasive autofluorescence of blood components has the potential to provide in vivo diagnosis of tumour stage, because cancerous tissues show enhanced fluorescence [7–10] of endogenous porphyrins as a consequence of tumour-specific metabolic alterations, with a high concentration of porphyrins synthesized and tumour hypervascularity.

Protoporphyrin IX is a porphyrin derivative that combines with ferrous iron to form the heme group [10]. Most iron in mammalian systems is routed to mitochondria to serve as a substrate for ferrochelatase. Ferrochelatase inserts iron into protoporphyrin IX to form heme, which is incorporated into hemoglobin (involved in the transport of oxygen). A number of non-heme iron-containing proteins are also known, such as the iron-sulphur proteins of oxidative phosphorylation and the iron transport and storage proteins transferrin and ferritin, respectively.

Tissue-specific regulatory features characterize the biosynthetic pathway of heme. In erythroid cells, regulation is mediated by erythroid-specific transcription factors and by the availability of iron in the form of Fe/S clusters. In nonerythroid cells, the pathway is regulated by heme-mediated feedback inhibition [10]. Heme biosynthesis starts in the mitochondria with the formation of δ-aminolevulinic acid (ALA), which involves the enzymatic condensation of glycine with succinyl-Coa. In the cytosol, two molecules of ALA form monopyrrole porphobilinogen through a condensation reaction catalyzed by aminolevulinate dehydratase (ALAD). The following step consists of the condensation of porphobilinogen into uroporphyrinogen III by the action of two enzymes, uroporohyrinogen I synthase and uroporphyrinogen III cosynthase. Uroporphyrinogen III is decarboxylated into coproporphyrinogen by the enzyme uroporphyrinogen decarboxylase. Coproporphyrinogen III is then transported to the interior of the mitochondria, where coproporphyrinogen III oxidase catalises the oxidative decarboxylation step from coproporphyrinogen III to protoporphyrinogen IX. Protoporphyrinogen IX oxidase converts protoporphyrinogen IX to protoporphyrin IX, which is responsible for the characteristic red color of heme. The final reaction in heme synthesis involves the insertion of the iron atom into PpIX, generating heme. The enzyme that catalyses this reaction is known as ferrochelatase [11].

While in nonerythroid cells the rate of heme synthesis depends on the rate of ALA production by the first and rate-limiting porphyrin biosynthetic enzyme, ALA-S, in erythroid cells it is determined by the availability of iron for ferrochelatase [12].

Protoporphyrin IX is the predominant porphyrin in blood [13]. Abnormal metabolism of protoporphyrin IX has been observed in total blood, plasma, serum and tissues of cancerous patients, which indicates that protoporphyrin IX accumulates substantially more in cancer cells than in normal cells and tissues [14–20].

Through the analysis of PpIX spectroscopic properties it is possible to monitor its concentration in tissues and biological fluids. The absorption spectra of porphyrin exhibit five bands: the Soret Band at around 400 nm and four bands, known as Q bands, in the region between 450 and 700 nm [6, 21].

Several studies can be found in the literature on the use of endogenous fluorescence for disease diagnosis [7–9, 14–20]. In this work we propose that the analysis of the intrinsic fluorescence of protoporphyrin IX from blood samples can yield information on the progression of prostate tumours. The aim of the current study is to search for a possible method to distinguish between normal specimens and those associated with prostate cancer in order to estimate the stages of induced tumour development in male NUDE mice, by examination with ultraviolet 405 nm irradiation.

Materials and methods

Cell line and cell culture conditions

"DU145 (DU-145) is a "classical" cell lines of prostatic cancer. The DU-145 is a prostatic human tumor cell line, established from the removed metastatic central nervous system tumour of an old man with prostate carcinoma in 1975 [22]. Du-145 cells were cultured in DMEM containing high glucose concentration (4.5 g/liter at 25 mM) and supplemented with 100 units/ml of penicillin, 50 mg/ml of

streptomycin, and 10% of FBS. The cells were maintained in a humid chamber at 37 °C in an atmosphere of 5% CO_2"

Animals and tumour induction

A total of five male NUDE mice, ~6 weeks old on arrival, were obtained from IPEN/CNEN-SP, 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. The orthotopic tumour model of prostate cancer was used, in which 1×10^5 cells were inoculated into the prostate gland in a volume of 0,1 mL of sterile phosphate buffered saline (PBS).

Blood sampling upon tumour growth

The animals were monitored and blood samples were collected before cells implantation (control animals) and at 7, 14, 21, 28 and 35 days after cells inoculation. Approximately 400 µL of blood was collected from the retro-orbital plexus of each animal with a glass capillary, using heparin as an anticoagulant. All experiments were performed in accordance with international guidelines for animal care.

Protoporphyrin IX calibration curve

Protoporphyrin Standard (*Sigma Porphyrin*. Products, Logan, Utah, USA) was dissolved in acetone (analytical purity) and solutions containing concentrations of 0.01, 0.25, 0.5, 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, and 10 µg/mL were prepared in triplicate. Emission spectra were obtained exciting samples at 402 nm. Average curves for each concentration were obtained and emissions between 580 and 780 nm were plotted as a function of protoporphyrin IX concentration to generate a calibration curve.

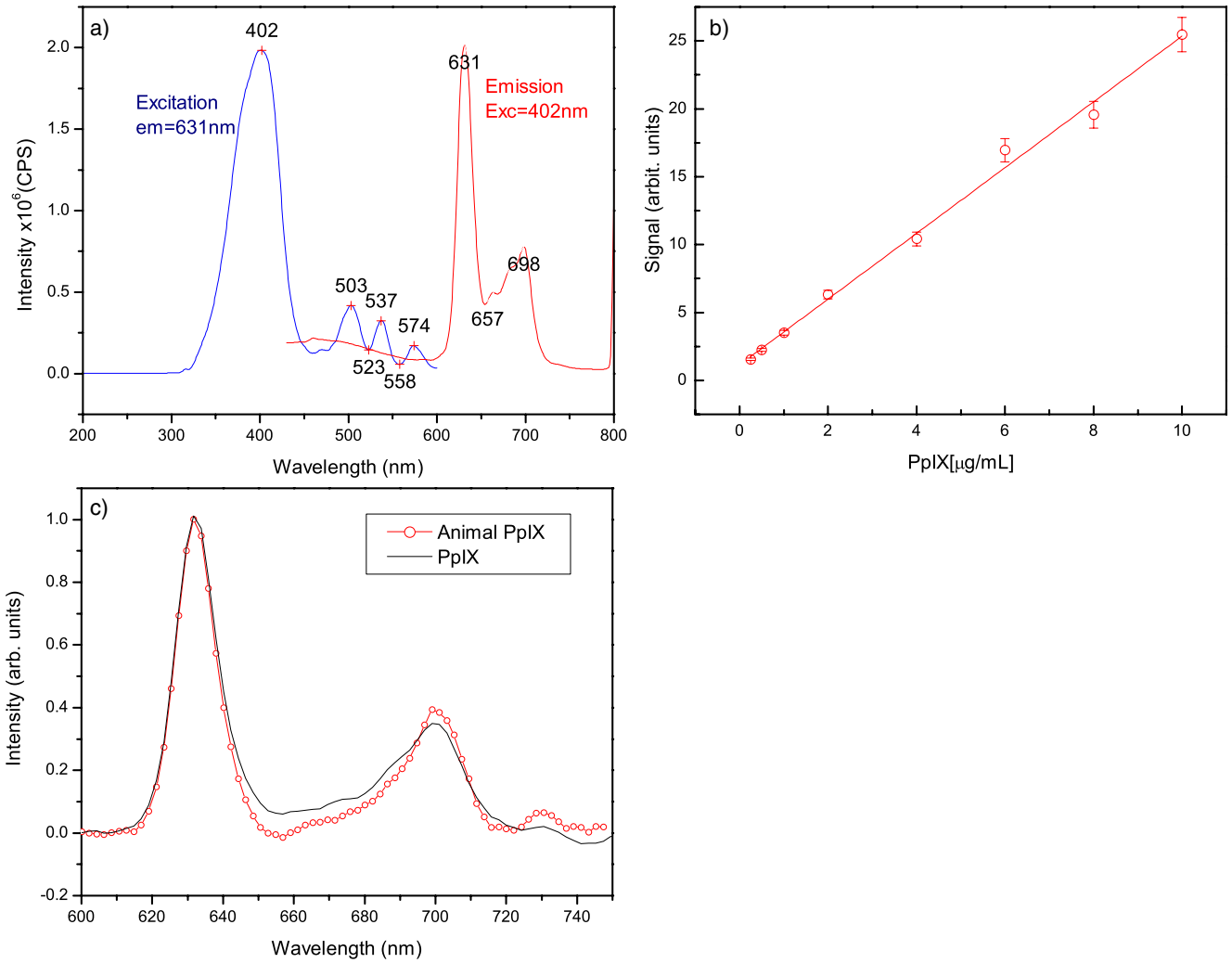

Fig. 1 **a** Excitation and emission spectra obtained for a 5 µg/mL PpIX acetone solution. **b** Protoporphyrin IX calibration curve. **c** Normalized emission spectra obtained exciting a 0.25 µg/mL protoporphyrin IX acetone solution and porphyrin extracted from the blood of normal mice at 402 nm

Porphyrin extraction

According to the standardized procedure used in reference [7], blood samples collected from the mice were centrifuged at 4,000 rpm for 15 min. The supernatant plasma was removed completely and three volumes of analytical grade acetone were added to the formed elements and mixed well. The mixture was centrifuged using the conditions described above. The clear supernatant of each mixture was collected in a clean tube and maintained at 4 °C before undergoing spectrofluorometer analysis.

Fluorescent spectral analysis

The emission spectra were obtained by exciting the samples inside a 1 mm optical path cuvette, using a modular homemade spectrometer with two arms arranged at 90°: an excitation arm and an emission arm. The excitation arm consisted of an excitation source, a 150 W Xenon lamp, a 0.25 m Jarrel Ash excitation monochrometer, a light mechanical modulator and a lens system that focused excitation light inside the cuvette. The emissions of the samples were analyzed by an emission arm containing 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. For emission measurements, excitation light was fixed at 405 nm and the emission monochrometor was turned up from 580 to 700 nm. In this case, spectral resolution was 0.5 nm. For excitation measurements, emission was fixed at 630 nm and the excitation monochromator was turned up from 200 to 500 nm. Spectral resolution for excitation measurements was 1 nm. No photobleaching was observed.

Tumour excision and histological analysis

On the 35th day, the five animals used in the experiment were sacrificed following the guidelines for euthanasia of the American Veterinarian Medical Association. Their prostates were excised and washed in PBS, fixed in 10% PBS-buffered formalin for 24 h, and then routinely processed for paraffin-embedding. Histological analyses were performed in 4 μm sections stained with hematoxylin and eosin.

Results and discussion

The starting point of this experiment was to verify the correlation between the increase in the fluorescence

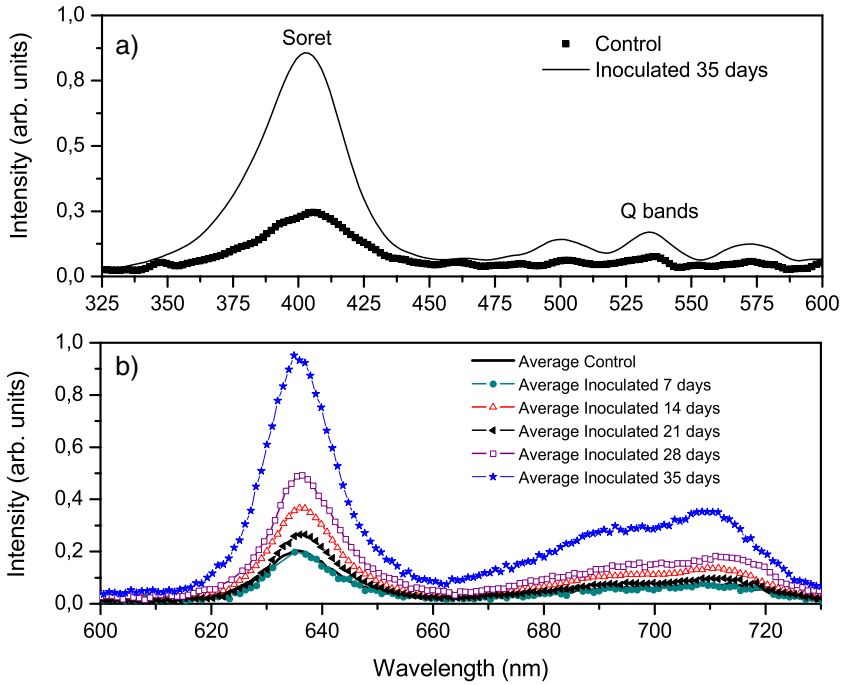

Fig. 2 **a** Average excitation spectra (emission at 630 nm) of porphyrin samples extracted from control and inoculated mice, indicating the Soret and Q bands. **b** Emission spectra obtained exciting samples of mice blood at 405 nm. Each curve corresponds to the average emission spectra of 5 animals, collected 7, 14, 21, 28, and 35 days after inoculation, and the control curve is an average of 3 animals. Using Student's *t* Test for comparison of difference in porphyrin fluorescence intensity between mice with and without tumor, the critical value of t for 8 degrees of freedom is 3.355 at a 99.5% confidence level. The calculated t value of 4.15 is higher than the critical value of 3.355. Therefore, at >99.5% confidence level we can conclude the results from control and tumor signals are significantly different

intensity signal of porphyrin extracted from the mice's blood using acetone and the progression of the prostate tumour. In order to quantify the porphyrin concentration we compared the emission spectra of normal animal blood protoporphyrin IX (PpIX) with PpIX acetone solution.

The excitation and emission spectra for a Sigma PpIX 5 μg/mL acetone solution are shown in Fig. 1. In the excitation spectrum, the Soret band around 402 nm can be clearly seen along with the three weaker Q bands at longer wavelengths (450 to 700 nm). The fluorescence spectrum with excitation at 402 nm is approximately mirror symmetric to the two long-wave absorption bands, and therefore it is natural to consider that the third band (at 574 nm) is a Q band while the 537 and 503 nm bands are its vibrational satellites.

In Fig. 1b we compare the emission spectra of a 0.25 μg/mL PpIX acetone solution, normalized by the intensity of the 631 nm band, with the one obtained from PpIX extracted from normal animal blood with acetone. A very similar emission profile can be observed, indicating that PpIX is the main factor responsible for the emission signal obtained in the blood. This fact is due to the extraction of blood porphyrin using acetone, which eliminates the signal of other molecular species. Consequently, light scattering from blood samples is negligible. This fact allows for the construction and use of a PpIX calibration curve, shown in Fig. 1c. To obtain this figure, the area measured below the porphyrin emission spectrum in the range of 580–780 nm is shown as a function of PpIX concentration, ranging from 0.01 to 10 μg/mL, and it can be seen that it increases linearly. A linear function was fitted to the experimental data do determine a calibration curve. Using the calibration curve presented in Fig. 1b, a normal blood porphyrin (control) concentration of 0.14 μg/mL could be obtained. Is important to mention that according to literature [23], there is no significant difference in the intensities or spectrum shape of PpIX fluorescence for variations in the pH of solutions.

The aim of the current study was to examine the correlation between porphyrin autofluorescence and tumour growth in order to search for a possible method to distinguish between normal and cancerous specimens. Therefore, blood porphyrin was extracted from the male NUDE mice in which the tumour was inoculated and excitation and emission spectra were obtained. The results are shown in Fig. 2. The excitation spectra of samples, obtained by fixing the emission at 630 nm for control and 35 days after tumour inoculation, are shown in Fig. 2a, which shows that emission intensity increases almost four times in sick mice. The estimated PpIX concentration in blood extracted 35 days after inoculation is 0.49 μg/mL.

Exciting the blood samples at 405 nm, the emission spectra of protoporphyrin IX, shown in Fig. 2b, were obtained. Each curve corresponds to an average of the 5 inoculated animals, and the control curve corresponds to 3 animals. In these spectra we observe the two characteristic bands of PpIX, centered on 635 nm and 700 nm. It can be clearly seen that the average spectrum increases in intensity as the tumour grows inside the animals, indicating that porphyrin is accumulating in their blood. The pPIX fluorescence signal remains constant for the uninfected NUDE mice.

To explain the progressive enhancement in the porphyrin spectra of inoculated mice we propose to correlate the abnormal action of hemoglobin in tumour tissues with alterations in tumour pH. Hemoglobin (Hb) in erythroid cells, as well as other proteins in other parts of the organism, acts as an important buffer that rapidly links to the excess of free hydrogen ions (H^+): $H^+ + Hb \Leftrightarrow HHb$. When the concentration of H^+ changes, the buffer systems of body fluids react to minimize these changes, not adding H^+ to nor removing it from the body, but holding it until balance can be re-established. Tumour tissues exhibit a

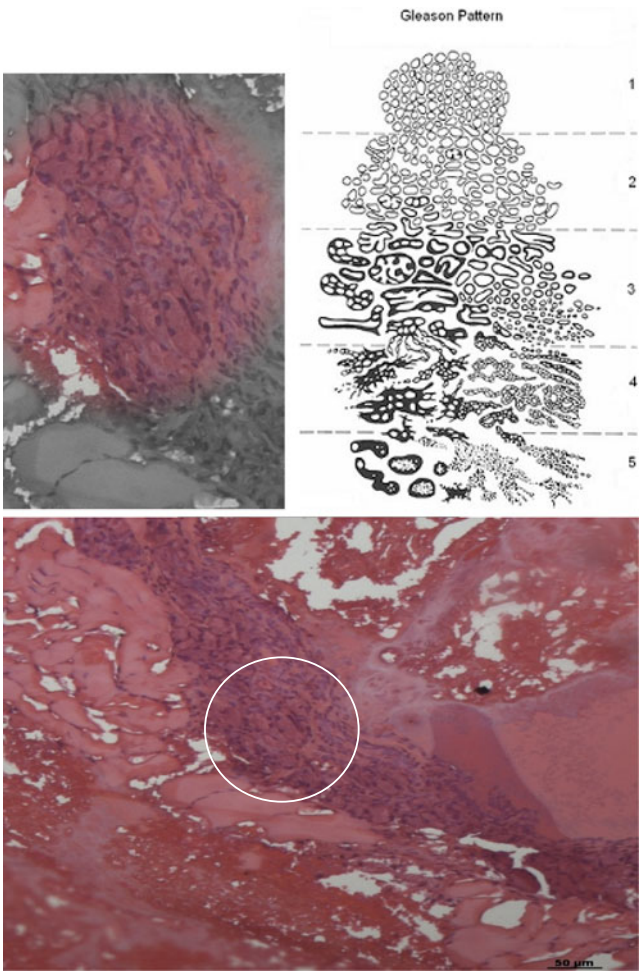

Fig. 3 Histological slide of the prostates of inoculated mice, compared with Gleason grade. Grade 3 tumour with individual glands arranged randomly (invasive), seen at low magnification

lower extracellular pH than normal tissues [24–26]; the production of lactic acid under anaerobic conditions and the hydrolysis of ATP in an energy-deficient environment is probably a major cause of this acidic microenvironment [27–29]. pH in healthy tissues is 7.4, and in tumours it is generally close to 6.9 [30].

Excessive production of hydrogen ions changes the ionization of molecules resulting from protonation or deprotonation. pH influences the rate of metabolic reactions, the behavior of biological membranes and membrane transport systems, the binding of molecules, and the actions and distribution of drugs, affecting the charge on proteins and other critical reactive groups.

Tumour hypervascularity enhances porphyrin fluorescence. As tumour tissues present hypervascularity, they contain more blood vessels then normal tissues; consequently, more hemoglobin reaches the tumour tissue, in an attempt at neutralization. In the cancer site, porphyrin is subjected to an acidic environment that may lead to protonation. This could be a decisive factor for the cellular retention of porphyrin in tissues and cells, associated with intracellular proteins, as a single molecule or agglomerate.

This enhancement of PpIX fluorescence proportional to the progression of cancer demonstrates that this technique can be used to determine tumour stage. A detailed experiment must be performed with the collected blood and the prostate gland, comparing the intensity of PpIX and the tumour site (pathology) to establish a link with Gleason score.

PpIX blood autofluorescence measurement can be performed to determine the effect of therapy and to monitor the treatment. The patient's blood autofluorescence, after successful cancer therapy, will show a level of PpIX similar to control blood.

In the third week, the concentration of PpIX decreased. This may have happened because erythrocytes have a life span of 43 days in healthy mice, and erythrocyte viability is affected by a large number of biochemical alterations, such as increased pH, increased lactate acid, decreased consumption of glucose, reduced ATP levels and loss of erythrocyte function.

On the 35th day the animals were sacrificed and their prostate glands were excised. Figure 3 shows the histological analysis. This picture shows, in the central region, an agglomerate of disorganized cells (purple color, related to cell nucleus), indicating the tumour. Figure 3 shows that the tissue still has recognizable glands, but the cells are darker, which is compatible with grade 3 tumours. Large and well-designed studies are under way to elaborate more on this matter.

It is important to mention that no inflammation was observed in animals and there are no other sources of PpIX elevation that are independent of the prostate cancer.

In the case of using porphyin testing in humans is important to observe that the blood is a very complex tissue and factors like effect of drugs, other diseases, as diabetes, gastric cancer, breast cancer, Hodgkin's lymphoma etc., would have important contribution to the enhancement of porphyrin concentration in the blood.

Conclusions

This study describes the autofluorescence of blood porphyrin as a marker for prostate tumors. We have used acetone extract of blood samples of NUDE mice and those infected with DU145 prostate cancer cell lines, to demonstrate that there was increase in porphyrin concentration in the blood samples of the infected mice. Exciting the blood samples at 405 nm we observed the two characteristic bands of PpIX, centered at 635 nm and 700 nm. It was evident that the intensities increased as the tumour grew inside the animals, indicating an accumulation of porphyrin in the blood. A normal blood porphyrin (control) concentration of a 0.14 μg/mL was obtained. The estimated PpIX concentration in blood extracted 35 days after inoculation is 0.49 μg/mL. To explain the progressive enhancement in the porphyrin spectra of inoculated mice, we propose to correlate the abnormal action of hemoglobin in tumour tissues with alterations in tumour pH. Fluorescence and excitation techniques appear to be useful for the diagnosis of tumour progression from its early stages. The study shows considerable evidence that these techniques could be used for the diagnosis of cancer at different stages, thus being a promising technique for mass screening at primary clinics. This type of "mass screening" could aid the early diagnosis of prostate tumours as a complementary testing for digital rectal examination, or PSA testing in the case of patients with inflammation of the prostate.

References

1. Kravchick S, Cytron S, Stepnov E, Ben-Dor D, Yakov K, Peled R (2009) 7 to 10 years' follow-up of 573 patients with elevated prostate-specific antigen (>4 ng/mL) or/and suspected rectal examination: biopsies protocol and follow-up guides. J Endourol 23:1007–1013
2. Miyakubo M, Ito K, Yamamoto T, Takechi H, Ohi M, Suzuki K (2009) Proprostate-specific antigen: its usefulness in the era of multiple-core prostate biopsy. Int J Urol 16:561–565
3. Mechergui YB, Ben Jemaa A, Mezigh C, Fraile B, Ben Rais N, Paniagua R, Royuela M, Oueslati R (2009) The profile of prostate epithelial cytokines and its impact on sera prostate specific antigen levels. Inflammation 32:202–210
4. Kvale R, Moller B, Wahlqvist R, Fossa SD, Berner A, Busch C, Kyrdalen AE, Svindland A, Viset T, Halvorsen OJ (2009) Concordance between Gleason scores of needle biopsies and

radical prostatectomy specimens: a population-based study. BJU Int 103:1647–1654
5. Salomatina E, Muzikansky A, Neel V, Yaroslavsky AN (2009) Multimodal optical imaging and spectroscopy for the intraoperative mapping of nonmelanoma skin cancer. J Appl Phys 105:102010
6. Scepanovic OR, Volynskaya Z, Kong CR, Galindo LH, Dasari RR, Feld MS (2009) A multimodal spectroscopy system for real-time disease diagnosis. Rev Sci Instrum 80:043103
7. Courrol LC, Silva FRD, Coutinho EL, Piccoli MF, Mansano RD, Vieira ND, Schor N, Bellini MH (2007) Study of blood porphyrin spectral profile for diagnosis of tumor progression. J Fluoresc 17:289–292
8. Kalaivani R, Masilamani V, Sivaji K, Elangovan M, Selvaraj V, Balamurugan SG, Al-Salhi MS (2008) Fluorescence spectra of blood components for breast cancer diagnosis. Photomed Laser Surg 26:251–256
9. Masilamani V, Al-Zhrani K, Al-Salhi M, Al-Diab A, Al-Ageily M (2004) Cancer diagnosis by autofluorescence of blood components. J Lumin 109:143–154
10. Ajioka RS, Phillips JD, Kushner JP (2006) Biosynthesis of heme in mammals. Biochim Biophys Acta-Mol Cell Res 1763:723–736
11. Hoggins M, Dailey HA, Hunter CN, Reid JD (2007) Direct measurement of metal ion chelation in the active site of human ferrochelatase. Biochemistry 46:8121–8127
12. Ponka P, Schulman HM (1993) Regulation of heme-biosynthesis—distinct regulatory features in erythroid-cells. Stem Cells 11:24–35
13. Ng JC, Qi LX, Moore MR (2002) Porphyrin profiles in blood and urine as a biomarker for exposure to various arsenic species. Cell Mol Biol 48:111–123
14. Bellini MH, Coutinho EL, Courrol LC, Silva FRD, Vieira ND, Schor N (2008) Correlation between autofluorescence intensity and tumor area in mice bearing renal cell carcinoma. J Fluoresc 18:1163–1168
15. Gibbs-Strauss SL, O'Hara JA, Hoopes PJ, Hasan T, Pogue BW (2009) Noninvasive measurement of aminolevulinic acid-induced protoporphyrin IX fluorescence allowing detection of murine glioma in vivo. J Biomed Opt 14:014007
16. Juzenas P, Juzeniene A, Iani V, Moan J (2009) Depth profile of protoporphyrin IX fluorescence in an amelanotic mouse melanoma model. Photochem Photobiol 85:760–764
17. Kleinpenning MM, Smits T, Ewalds E, van Erp PEJ, van de Kerkhof PCM, Gerritsen MJP (2006) Heterogeneity of fluorescence in psoriasis after application of 5-aminolaevulinic acid: an immunohistochemical study. Br J Dermatol 155:539–545
18. Larsen ELP, Randeberg LL, Gederaas OA, Arum CJ, Hjelde A, Zhao CM, Chen D, Krokan HE, Svaasand LO (2008) Monitoring of hexyl 5-aminolevulinate-induced photodynamic therapy in rat bladder cancer by optical spectroscopy. J Biomed Opt 13:044031
19. Lu SJ, Chen JY, Zhang Y, Ma J, Wang PN, Peng Q (2008) Fluorescence detection of protoporphyrin IX in living cells: a comparative study on single- and two-photon excitation. J Biomed Opt 13:024014
20. Utsuki S, Miyoshi N, Oka H, Miyajima Y, Shimizu S, Suzuki S, Fujii K (2007) Fluorescence-guided resection of metastatic brain tumors using a 5-aminolevulinic acid-induced protoporphyrin IX: pathological study. Brain Tumor Pathol 24:53–55
21. Uttamlal M, Holmes-Smith AS (2008) The excitation wavelength dependent fluorescence of porphyrins. Chem Phys Lett 454:223–228
22. Stone KR, Mickey DD, Wunderli H, Mickey GH, Paulson DF (1978) Isolation of a human prostate carcinoma cell line (DU 145). Int J Cancer 21:274–281
23. Piot B, Rousset N, Lenz P, Eleouet S, Carre J, Vonarx V, Bourre L, Patrice T (2001) Enhancement of delta aminolevulinic acid-photodynamic therapy in vivo by decreasing tumor pH with glucose and amiloride. Laryngoscope 111:2205–2213
24. Kozin SV, Shkarin P, Gerweck LE (2001) The cell transmembrane pH gradient in tumors enhances cytotoxicity of specific weak acid chemotherapeutics. Cancer Res 61:4740–4743
25. Chu GL, Dewey WC (1988) The role of low intracellular or extracellular pH in sensitization to hyperthermia. Radiat Res 114:154–167
26. Gerweck LE, Seetharaman K (1996) Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. Cancer Res 56:1194–1198
27. Newell KJ, Tannock IF (1989) Reduction of intracellular-pH as a possible mechanism for killing cells in acidic regions of solid tumors—effects of carbonylcyanide-3-chlorophenylhydrazone. Cancer Res 49:4477–4482
28. Tannock IF, Rotin D (1989) Acid pH in tumors and its potential for therapeutic exploitation. Cancer Res 49:4373–4384
29. Tannock IF (1989) Environmental-factors within solid tumors that influence cell-growth and viability. Cancer Metastasis Rev 8:146–146
30. Kubat P, Lang K, Anzenbacher P (2004) Modulation of porphyrin binding to serum albumin by pH. Biochim Biophys Acta-General Subjects 1670:40–48