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ARTICLE

Spectrofluorimetric Detection of DMBA-Induced Mouse Skin Carcinoma

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An attempt has been made to evaluate the normal and cancer blood samples of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mouse skin carcinoma by spectrofluorimetric method. Analysis of acetone extracts of plasma, erythrocyte and erythrocyte membrane showed an alteration around 630 nm when excited at 400 nm by cancer samples, compared to normal samples. The ratio of fluorescent intensity at 530 nm/630 nm was found to be decreased in erythrocyte and plasma and increased in erythrocyte membrane. These changes are not detectable in both hemolysates. It has been suggested that erythrocytes may be the carriers of fluorophors that accumulate in cancer tissue and may be useful in the diagnosis and treatment of malignancies. (Pathology Oncology Research Vol 5, No 1, 46–48, 1999)

Key words: mouse skin carcinoma, plasma, erythrocytes, erythrocyte membrane, fluorescent intensity, fluorophors

Introduction

In recent years optical spectroscopy become the basis for intensive research activity towards the development of novel non-invasive technique for tissue diagnosis. The specific optical spectrum of a tissue sample contain information about the biochemical composition and/or the structure of the tissue, both of which undergo a change during malignant transformation. These changes are detectable as an alteration in the fluorescent spectral profile of the tissues.[4] Previously, we utilized this technique for the discrimination of malignant stage from normal and premalignant stages during Ocimum sanctum Linn. treatment on hamster buccal pouch carcinogenesis.[5] Though tissues have been widely studied for autofluorescence, characterization of blood samples have not been clearly studied.[7,8] We made an attempt to study the fluorescence of blood samples in skin carcinoma bearing mouse model to discriminate them from their respective normals using fluorescent spectroscopy.

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Materials and Methods

Thirty six young male Swiss albino mice of Wistar strain weighing 18–22 g (purchased from King Institute of Preventive Medicine, Madras, India) were maintained on a commercial diet (Brooke Bond Lipton India Ltd, Calcutta, India) and water ad libitum. All the animals were dorsally shaved by surgical blade. Tumors were found to be well differentiated squamous cell carcinoma with prominent vascularity. When the tumors were of >15 mm diameter (the size of the tumor may not have an impact on the fluorescence status once it is become malignant) the animals were ether anesthetized and blood was collected with EDTA from eye vein using heparinized capillary tube. The blood was centrifuged at 3000 rpm for 5 min to seperate plasma. The pelleted elements washed with 0.9% saline and centrifuged at 3000 rpm, and the procedure was repeated thrice to seperate erythrocytes. A known amount of erythrocytes were lysed with ice cold distilled water for 60 min at 0°C. This was centrifuged at 15,000 rpm for 20 min to separate the erythrocyte membrane. To the known volume of supernatant (hemolysate), pale yellow to white pellet (erythrocyte membrane), plasma, and erythrocyte, 2 ml of analytical grade acetone was added. This was vortexed and centrifuged at 5000 rpm for 10 min. The clear super-

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natants (acetone extracts) were subjected to fluorescent spectral analyses at 400 nm excitation using a spectrofluorometer (Kontron, SFM-25, USA) and the emission spectra were scanned from 430 nm to 700 nm. The experiments were repeated thrice and the data obtained were statistically analyzed using Students' t test.

Results and Discussion

Autofluorophors or fluorophors of native tissues are characteristics of a given tissue and any alteration in the pathological status can be sensitively detected using fluorescent spectroscopy.[10] Since it is well known that tumor angiogenesis takes place during the proliferation,[2] it is thought that the transport of these fluorophors may be possible and can be detected in blood erythrocytes.

The normal and cancer samples produced their unique behaviour in the emission spectra on excitation at 400 nm. The fluorescent spectra and their emission characteristics were given in *Figure 1.* and *Table 1,* respectively. The normal plasma and erythrocyte showed a prominent peak at ~430 nm and decreases at longer wavelengths as shown in *Figure 1*. The erythrocyte membrane of both samples showed a maximum intensity at ~440 nm and a second peak at ~630 nm while erythrocyte membrane of the cancer sample also showed a secondary peak at ~540 nm. The cancer plasma and erythrocytes showed a peak at ~630 nm, while their respective normals did not show a peak at ~630 nm. The acetone extracts of cancer erythrocytes fluoresce more than its plasma in terms of intensity (FI). This suggests that there is a definite relationship between the erythrocytes and the fluorophores at ~630 nm. The hemolysate of normal and cancer samples behave similarly and are comparable with normal plasma and erythrocytes (spectra not given).

Table 1. **Emission characteristics of plasma, erythrocyte, erythrocyte membrane of cancer and normal blood samples of mouse excited at 400 nm**

Status	*Ratio of fluorescent intensity at 530nm/630nm (FI 530nm / 630nm)*
Normal plasma	2.00 ± 0.02
Cancer plasma	0.67 ± 0.03*
Normal erythrocytes	2.51 ± 0.05
Cancer erythrocytes	0.25 ± 0.02*
Normal erythrocyte membrane	1.15 ± 0.01
Cancer erythrocyte membrane	1.4 ± 0.03*

Mean ± SD for six animals in each group.
*p<0.001, when compared to respective normal samples.

For peaks at 470 nm, 520 nm and 630 nm, NAD(P)H, various flavins and porphyrins are responsible, respectively. Flavin and porphyrin fluorescence in the neoplastic tissues were lower or higher than the normal tissue depending on their nature. In the present study the ratio of fluorescent intensity at 530 nm/630 nm was found to be significantly decreased (p<0.001) in plasma and erythrocytes of cancer samples and erythrocyte membranes showed an increase (p<0.001) when compared to normal samples. The increase in the ratio of fluorescent intensity at 530 nm/630 nm in erythrocyte membrane and decreased ratio in the plasma and erythrocyte may be due to the alterations in the ratio of flavin/porphyrin contents in the cancer samples compared to normal. We found that the ratio of fluorescent intensity at 530 nm/630 nm in cancer plasma and erythrocytes is below one. The spectra observed in the cancer plasma and erythrocytes and the fluorophors present in the erythrocyte membrane of both samples found to

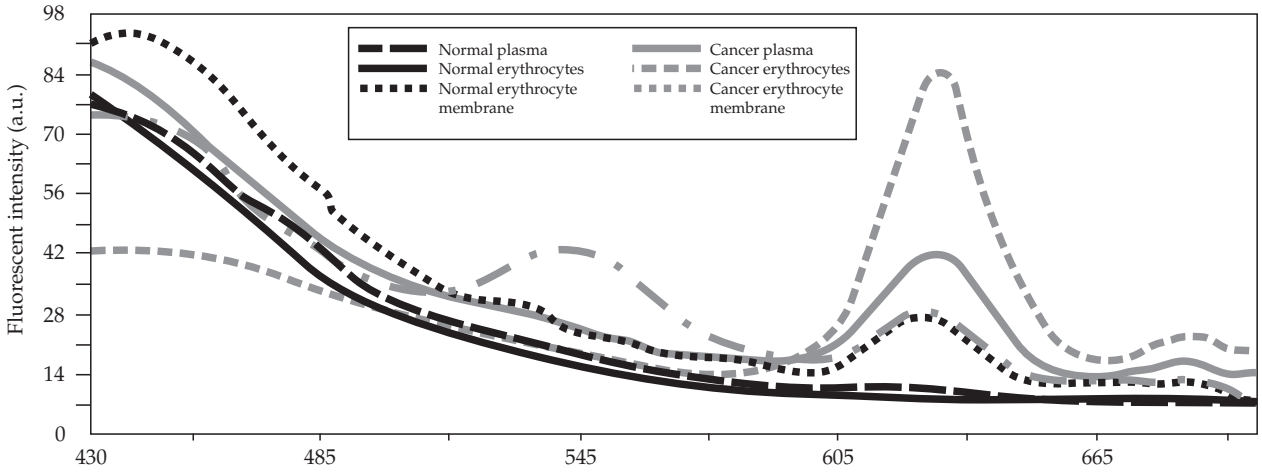

Figure 1. *Emission spectra of plasma, erythrocytes, erythrocyte membrane from normal and cancer samples on excitation at 400 nm.*

have a close agreement with the fluorophors that accumulated in the cancer tissue regarding 630 nm peak as reported by other researchers.[1,6] In all these studies, there was a clear indication that the peak around 630 nm is associated with carcinomatous status apart from that seen in erythrocyte membrane. Yang et al[9] reported that porphyrin compounds showed characteristic autofluorescence at 630 nm and 690 nm and suggested that they may be from the degradation of hemoglobin. Since this is possible, the degradation of hemoglobin may contribute for the hike in the porphyrin content (hence, 630 nm peak). But Balasubramanian et al[1] reported that these porphyrins may be of endogenous origin, due to the changes in the cellular environment. It is not known at present whether the cellular changes induce the production of fluorophors locally in the tumor environment or elsewhere in the host. The changes in the autofluorescence of the hamster buccal pouch mucosa can be detected as early as 4–6 week following application of DMBA.[1,9]

Since the present study was a preliminary investigation of the experimental carcinoma, we are yet to standardize the systematic changes in the fluorescent spectral profile of blood in relation to spontaneous as well as induced tissue transformation. We suggest that the erythrocytes may be the carrier of these kind of fluorophors either from the cancer tissues or to them. The relationship of erythrocytes with these fluorophors in the cancerous status may definitely provide clues whether they can be used as carriers of photosensitizer in the photodynamic therapy of malignancies. As there are number of reports for autofluorescence of tissue diagnosis that have been published,[3,4] we report that erythrocytes and plasma can also be used for the diagnosis of malignancies and the ratio of FI 530 nm/630 nm can be taken as a variance, which when below one (<1.00), could indicate carcinomatous status. Though the present work has been carried out in blood samples, further studies on other body fluids can be considered.

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