

## Protoporphyrin and Zinc Protoporphyrin in the Blood of Tumor Transplanted Mice

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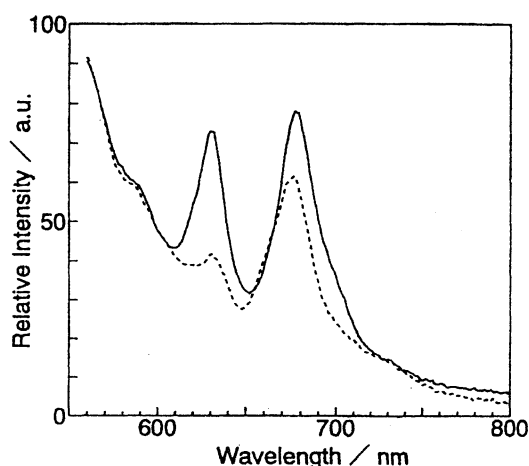
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Fluorescence at 630 nm was detected from the blood of tumor implanted mice. The fluorescence was attributed to protoporphyrin and zinc protoporphyrin. The fluorescence intensity increased with the increase of the period from tumor implantation and then decreased. By measuring the fluorescence intensity of the blood, the progress of cancer will be followed.

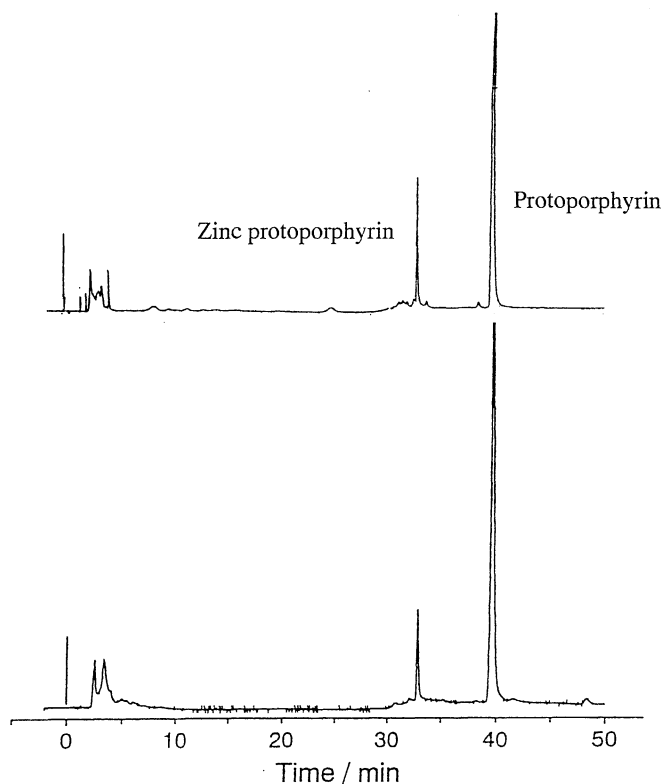
The fluorescence with the wavelength at around 630 nm have been detected from human abdominal cancer and mouse solid tumor.<sup>1,2</sup> In the solid tumors, the accumulation of the porphyrins with fluorescence has been proposed. In this study, the measurement of fluorescence from the blood of the mice with solid tumors was carried out, and the pathway of the production of the fluorescent compounds was discussed.

MH134 mouse tumor cells were implanted in C3H/HeN mice (Jcl Lot No. 28-35, weight:  $19.0 \pm 0.9$  g). After the implantation of MH134 tumor cells, the blood of the mice were collected at prescribed time intervals. Then the blood was centrifuged ( $2000 \times g$ ) for 10 min at  $4^\circ\text{C}$  to remove the corpuscles and the plasma was obtained. To extract the fluorescent compounds the plasma was treated with ethyl acetate - acetic acid (4:1, v/v) mixed solvent, and the extract was analyzed by HPLC with ODS column. Fluorescence spectra were measured by using Hitachi F-850 spectrometer with Toshiba Y-43 filter.

Figure 1 shows a typical fluorescence spectrum from the plasma of tumor implanted mouse. At 630 nm a characteristic fluorescence band appeared, and the fluorescence spectrum was



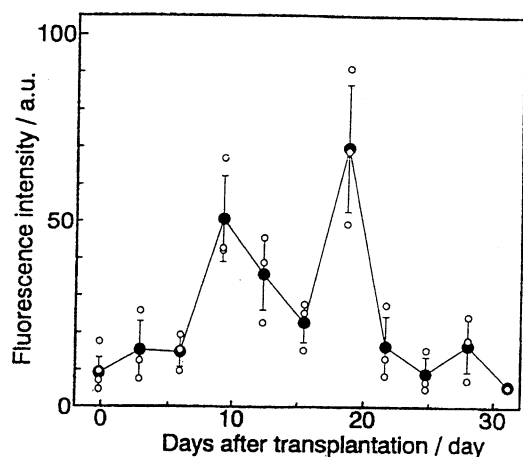
**Figure 1.** Fluorescence spectra from the plasma of tumor implanted mouse (solid line) and the plasma of normal mouse (dotted line).



**Figure 2.** HPLC chromatogram from plasma (a) and solid tumor (b) in tumor implanted mouse.

almost the same as that obtained from the extract of the mouse solid tumor. As shown in Figure 2, by HPLC analysis of the extract of the plasma, identification of protoporphyrin and zinc-protoporphyrin was established. In this figure HPLC chromatogram of the extract of solid tumor is also shown. As these porphyrins show a characteristic fluorescence band at 630 nm, these fluorescent compounds were identified as protoporphyrin and zinc-protoporphyrin. By measuring the fluorescence spectra at 630 nm, the mice with tumor can be identified. Protoporphyrin is an intermediate in the heme biosynthesis and it is not contained in the blood of normal mouse. Protoporphyrin in tumor implanted mouse may be accumulated by inhibition of iron insertion to protoporphyrin, and/or the extract of the iron from heme protein by abnormal metabolism. Zinc-protoporphyrin may also be formed by the insertion of zinc ion to protoporphyrin.

Time dependence of fluorescence intensity of the blood of the tumor implanted mouse was measured. The fluorescence from the blood of normal mouse was scarce. Figure 3 shows the



**Figure 3.** Time dependence of fluorescence intensity at 630 nm from the plasma of tumor implanted mouse.

fluorescence intensity with period from tumor implantation. The fluorescence intensity increased with the period from tumor implantation and then the intensity decreased. Strong fluorescence intensity was measured when the tumor cells were active from 7 to 20 days after implantation. When the fluorescence intensity decreased the tumor became large enough and the mice were dying. When the tumor cells were almost dying after 20 days, the fluorescence intensity also decreased. From the above results, by measuring the fluorescence intensity of the blood, the degree of the progress of cancer will be followed.

**Table 1.** Porphyrin Formation with Various Cell Strains

Strain	Protoporphyrin <sup>a</sup>	Zn-Protoporphyrin <sup>a</sup>
HeLa	$4.9 \times 10^{-10}$ M	$1.0 \times 10^{-10}$ M
Hepatoma	$1.6 \times 10^{-10}$ M	$0.9 \times 10^{-10}$ M
Fibroblast (bone marrow)	$0.3 \times 10^{-10}$ M	0
Fibroblast (amniotic fluid)	0	$0.1 \times 10^{-10}$ M

<sup>a</sup> Porphyrin concentration was measured after 3 days cultivation (cell number:  $5 \times 10^5$  cells/ml).

As shown in Table 1, we also detected protoporphyrin and zinc protoporphyrin from the cultivation medium of tumor cells such as HeLa and Hepatoma II cells. However, these porphyrins were hardly produced from normal cells such as Fibroblast (from Bone marrow and Amniotic fluid). Therefore the production of these porphyrins may be characteristic for tumor cells. The study to clarify the reaction mechanism of these porphyrins formation is now in progress.

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#### References and Notes

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