EPR effect in metastatic and autochthonous tumor and antimetastatic effect of SMA-pirarubicin (THP)

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ABSTRACT SUMMARY
The present research confirmed the EPR effect exists in the metastatic tumors and primary tumor in the colon or the breast both are chemically induced autochthonous tumors in vivo. SMA-pirarubicin micelles showed tumor suppression in the metastatic tumor nodules in the lung, remarkably, as was in primary tumors.

INTRODUCTION
The EPR (enhanced permeability and retention effect) of solid tumor is now well established. However, demonstrations of EPR effect are observed mostly in the experimentally implanted tumors in rodents. In human clinical setting, it is observed in hepatoma, renal cancers and others when SMANCS/Lipiodol (lipid contract agent) was infused into the tumor feeding artery, or by radioscintigraphy using radioactive \(^{67}\text{Ga}\) given iv, where radioactive gallium would bind to transferrin (becoming 90KDa), thus EPR dependent tumor staining is seen\(^{(1,2)}\).

Here we report fluorescent imaging of metastatic and autochthonous tumor models induced by azoxymethan (AOM) plus Na dextran sulfate (DSS) given ip and po respectively for colon cancer in mice, or by DMBA given po once for breast cancer in rats, respectively.

Antimetastatic effect using polymer conjugated pirarubicin (tetrahydropyranyl doxorubicin, THP) was examined using lung metastasis of colon cancer model in mice.

EXPERIMENTAL METHODS
Fluorescent nanoprobe of poly-HPMA-ZnPP (Zn-protoporphyrin), rhodamine-labeled albumin and SMA-indocyanine green were prepared as described\(^{(1-3)}\).

We also prepared SMA-THP conjugate as described in the separate paper in this CRS Meeting (Tsukigawa K, Nakamura H, Liao L, Fang J, Maeda H, et al), and examined antitumor effect of SMA-THP using colon 26 mouse tumor. The colon 26 tumor cells (\(1 \times 10^6\) cells per site) were implanted on the dorsal skin bilaterally in Balb/c mice. Drug was injected into the tail vein at the dose of 10 mg/kg and 30 mg/kg, on day 10 after tumor inoculation when tumor size reached to about 5-6 mm in diameter.

Another experiment is to see the accumulation of HPMA-ZnPP micelle and rhodamine-albumin conjugate (micelle) in the primary breast cancer induced by DMBA in SD rats\(^{(4)}\), and colon cancer induced by AOM (ip) followed by DSS (po) in the drinking water for 1 week. Metastatic tumor in the lung was obtained by colon 26 carcinoma implanted sc in the dosal skin and 4 weeks after tumor inoculation the effect of SMA-THP on the metastatic lung cancer was examined.

RESULTS AND DISCUSSION
Evaluation was carried out using above fluorescent nano-probes under the IVIS in vivo imaging system. All cases showed fluorescent tumor image similar to implanted tumors. Namely, DMBA and AOM/DSS induced autochthonous breast cancer in rats and colon cancer in mice respectively showed tumor image similar to implanted tumor seen in S-180 and colon 26. Image of metastatic tumor nodules in the lung of colon 26 tumor model also showed clear fluorescence.
More important, antitumor effect of SMA-THP micelles on the lung metastasis of colon cancer showed clear tumor suppression similar to the implanted primary colon 26 tumor.

These results shows the EPR effect operates not only implanted but also in the autochthonous tumor, as well as metastatic tumor nodules. These results are consistent to our previous finding that polymer conjugate (SMANCS) exhibited highly tumor tropic nature than native drug\(^{5-7}\). More important is that these nanomedicines have not only important in selective tumor uptake but also in the metastatic tumor nodules, and provide imaging potential based on the EPR effect.

![Figure 1. Metastatic lung cancer of colon 26 carcinoma from dorsal skin. A. No drug control showed numerous tumor nodules in the lung. B. Lung metastasis was significantly decreased after SMA-THP micelle treatment. (cf. A and Table 1).](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Metastasis score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0/5</td>
</tr>
<tr>
<td>Given iv, on day 10 once only</td>
<td>10</td>
<td>2/5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5/5</td>
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**REFERENCES**


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