

## **Research of Photodynamic diagnosis by using photosensitizer Talaporphin-sodium for lymph node metastasis.**

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### **Abstract**

Photodynamic diagnosis (PDD) is the new diagnosis methods for cancer using light and photosensitizer. Among them, second-generation photosensitizer Talaporphin-sodium especially has high therapy effect for cancer. Therefore, Talaporphin is expected to be also applied to PDD. In this study, we measured to effectiveness of PDD for lymph node metastasis by means of mouse lymph node metastasis model. Mouse model was made using human colon cancer cell line HT29 and green fluorescent protein (GFP). Photosensitizer Talaporphin-sodium was administered at a final concentration of  $5 \cdot 10$  mg / kg at before experiment  $6 \cdot 12$  hours and Indocyanine green (ICG) was administered at a final concentration of 5 mg / kg at before experiment 10minutus. The irradiated lasers are 405 nm, 664 nm for Talaporphin, 488 nm for GFP and 785 nm for ICG respectively. The results of fluorescence spectrum were confirmed that high fluorescence spectrum of 664 nm in lymph node, and fluorescence of GFP gene transfected into tumor cells was also observed. Moreover, results of imaging were overlapped fluorescence of ICG and Talaporphin, indicated the effectiveness in imaging. From these results, the diagnostic method combining Talaporphin and ICG showed efficacy against lymph node metastasis.

Key words: Talaporphin-sodium, photodynamic diagnosis, laser diode, green protein, lymph node metastasis

### **Introduction**

Photodynamic diagnosis and photodynamic therapy (PDT) using photosensitizer are highly effective in various carcinomas. The second-generation photosensitizer, Talaporphin-sodium, has already been successfully used in PDT of brain tumor, early lung cancer and esophageal cancer. Therefore, it is also

expected to be effectively used in PDD on peritoneal dissemination and lymph node metastasis. In this study, we aim to develop a mouse lymph node metastasis model, by measuring the fluorescence imaging and spectrum thereby to establish an effective PDD diagnosis.

## Material and Method

The human colon cancer cell line HT29 was used to generate mouse lymph node metastasis model. The HT29 cell line was transfected into six mice with GFP. The GFP was transformed by incubating the retroviral vector with HT29 for 24 hours. Also, GFP were used as marker for examining the presence or absence of lymph node metastasis. Talaporfin was intraperitoneally injected at a dose of 5, 10 mg / kg body weight and, elimination time after administration was 6 or 12 hours. Moreover, lymph nodes were identified using ICG for lymph node detection. The new laparoscopic system was used for the measurement device, and presence or absence of lymph node metastasis was detected by fluorescence spectrum and fluorescence imaging. of 405 nm and the 664 nm laser diodes were excited for Talaporfin-sodium, The 488 nm laser diode was excited for GFP, and 785 nm laser diode was excited for ICG. A total of 20 mouse models were generated and divided into 8 groups. 3 groups 6 animals of lymph node metastasis and 2 animals of non-lymph node metastasis.

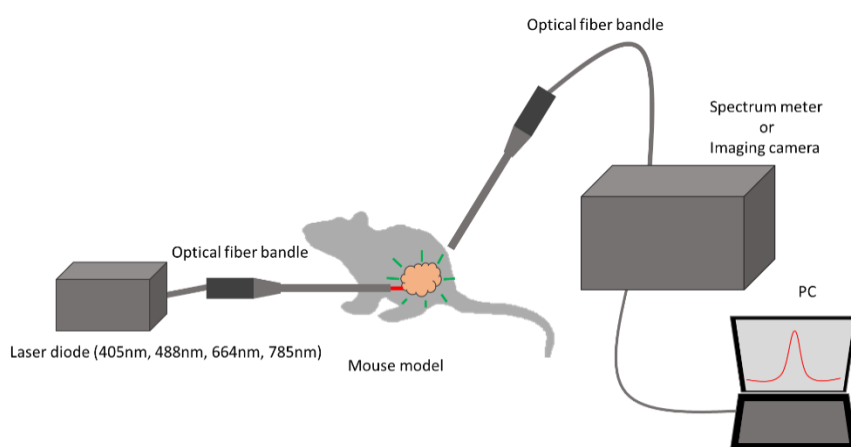


Fig.1 experiment system. The results of fluorescence spectrum was suggested using optical fiber bundle and spectrum meter. Similarly, results of fluorescence imaging was suggested using optical fiber bundle and imaging camera.

## Results and Discussion

As a result of the fluorescence spectrum in lymph node metastasis model, 16 of 18 animals were able to measure fluorescence at 670 nm of Talaporfin and fluorescence at 785 nm of ICG. Thereby the effectiveness of the diagnosis lymph node metastasis was show. The result of the fluorescence image overlapped the position of the fluorescence image of Talaporfin and the fluorescence image of ICG, but it was rare that it overlapped with the fluorescence image of GFP. When the tumor tissue was deep,

fluorescence of long wavelength Talaporfin and ICG was transmitted. However, it is considered that GFP was not transmitted because of its short wavelength.

These results seem to indicate the effectiveness of a new detection method for lymph node metastasis. In addition, it was suggested that high precision PDD is possible by combining fluorescence spectra and fluorescence imaging.

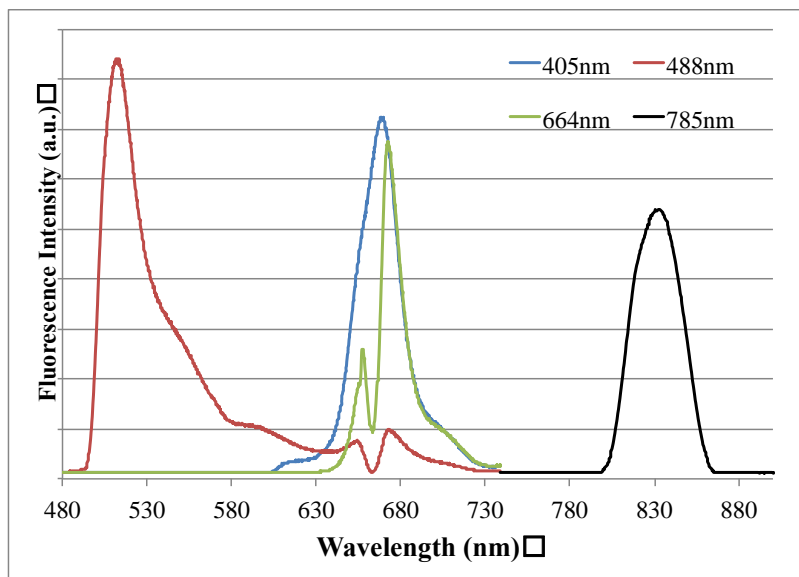


Fig.2 The fluorescence spectrum of Talaporfin administered after 12 hours. The red line is the Fluorescence spectrum of GFP due to excitation light at 488 nm. The blue line is the Fluorescence spectrum of Talaporfin due to the excitation light at 405 nm. The green line is the Fluorescence spectrum of Talaporfin due to excitation light at 644 nm. The black line is the Fluorescence spectrum of ICG due to excitation light at 785 nm.

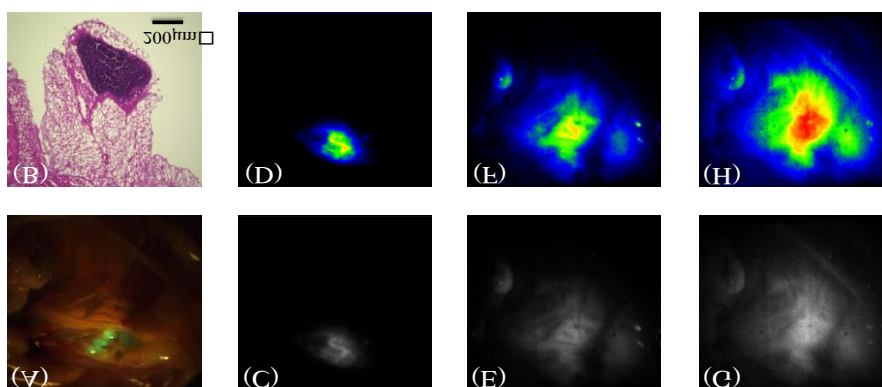


Fig.3 The fluorescence imaging of mouse model after 12 hours administration of Talaporfin. (A) Visible light imaging of lymph node excited by LED light. (B) H&E stain in lymph node. (C), (E), (G) IR imaging were irradiated for lymph node by a laser diode each excitation light, 405nm (C), 664nm (E), 785nm (G). (D), (F), (H) Color temperature imaging were processed from each IR imaging of lymph node.

## **Reference**

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