

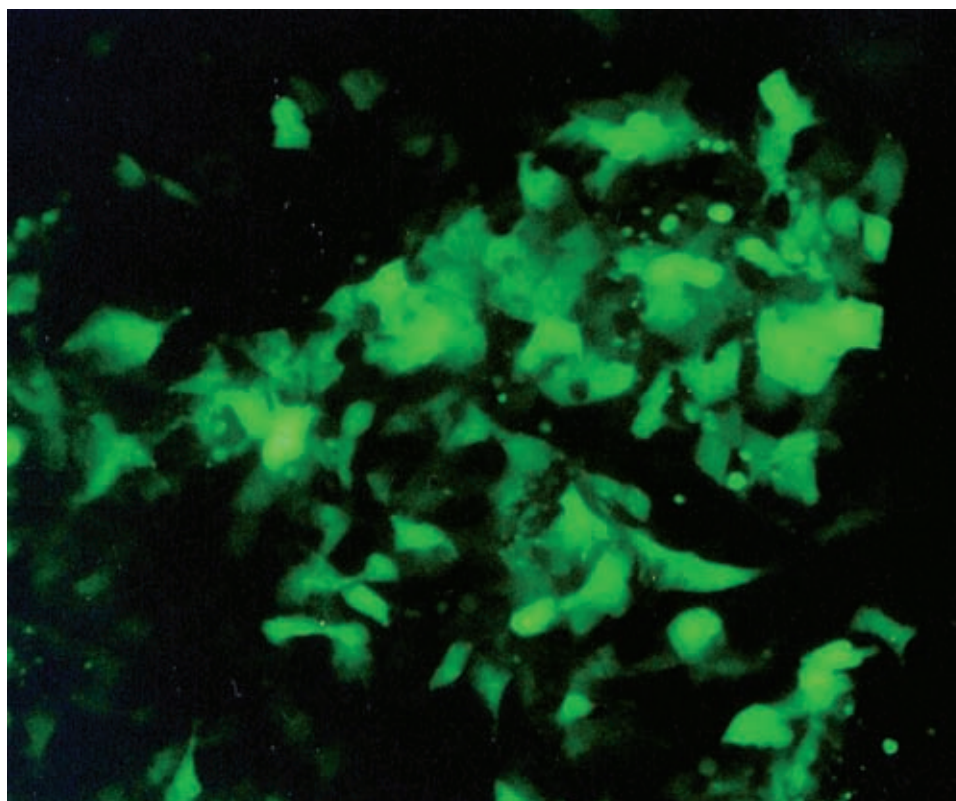
—Photogravure—

## External Whole-body Image of EGFP Gene Expression

Seiichi Shinji<sup>1,2</sup>, Toshiyuki Ishiwata<sup>2</sup>, Takashi Tajiri<sup>1</sup>, Noritake Tanaka<sup>1</sup>, Tomoko Seya<sup>1</sup>,  
Kiyoko Kawahara<sup>2</sup>, Munehiro Yokoyama<sup>2</sup> and Zenya Naito<sup>2</sup>

<sup>1</sup>Department of Surgery I, Nippon Medical School

<sup>2</sup>Department of Pathology II, Nippon Medical School



**Fig. 1** Stable, high-level GFP-expressing HCT-15 human colon cancer transfectant in vitro (Fluorescence microscopy,  $\times 400$ ).

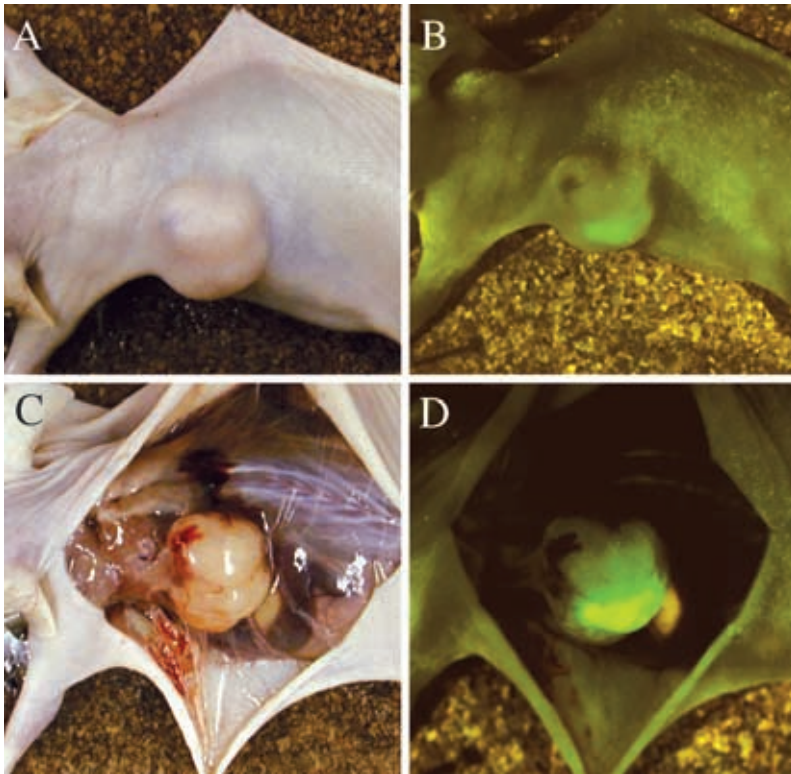
### Abstract

Whole-body optical imaging<sup>1-3</sup> is an external and noninvasive procedure that enables the continuous visual monitoring of malignant growth and spread within intact animals. The human colon adenocarcinoma cell-line HCT-15 was transfected with a pIRES 2-EGFP vector, and stable enhanced green fluorescence protein (EGFP) expression was established (**Fig. 1**). Approximately  $10^6$  HCT-15 EGFP stable transfectant cells were subcutaneously injected into the left flank of six-week-old male Balb/c-nu/nu mice. On post-injection day 78, the size of the subcutaneous tumor was 13.1 mm  $\times$  15.4 mm in diameter, as observed using an ORCA-C 7780-20 three-chip cooled color charge-coupled-device camera (Hamamatsu Photonics Systems, Hamamatsu City, Japan). An external fluorescent image of the tumor was acquired through the skin (**Fig. 2B**). The tumor could be seen more clearly once the skin was removed (**Fig. 2D**). Furthermore, in the peritoneal metastasis (**Fig. 3B**) and liver metastasis models (**Fig. 3D**), metastasis could also be seen through the skin. This new technology is a useful method for investigating tumor growth in vivo. In the future, this method could be applied to the detection of peritoneal, liver and lung metastasis in living animals.

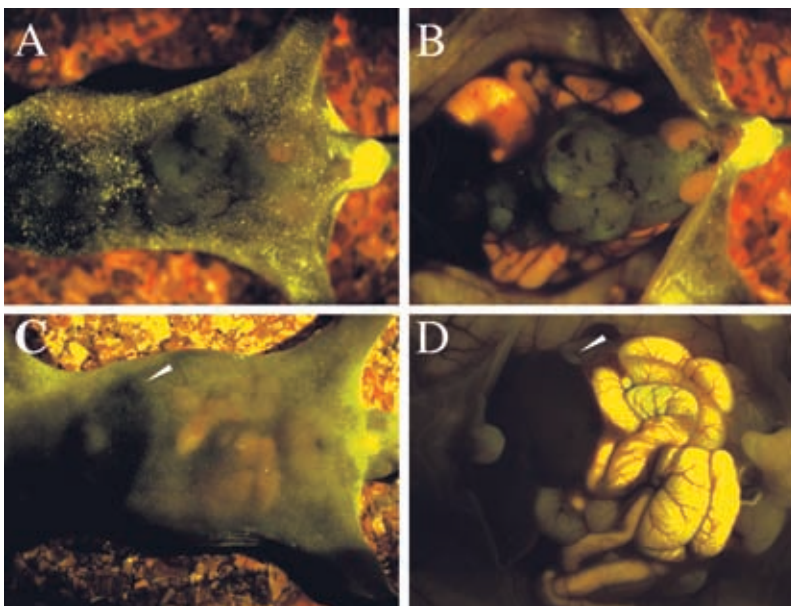
Correspondence to Seiichi Shinji, MD Department of Surgery I, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan

E-mail: s-shinji@nms.ac.jp

Journal Website (<http://www.nms.ac.jp/jnms/>)



**Fig. 2** External whole-body image of EGFP expression in a subcutaneous tumor. The tumor can be visualized through the skin (B). After removing the skin, the tumor was more clearly visualized (D) (B, D: ORCA-C7780-20 3CCD camera).



**Fig. 3** External whole-body image of EGFP expression in the peritoneal cavity (A, C) and its direct image (B, D). Large peritoneal dissemination (B) and liver metastasis (C, arrowhead) could be visualized through the skin.

**References**

1. Hasegawa S, Yang M, Chishima T, et al: In vivo tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. *Cancer Gene Ther* 2000; 7: 1336-1340.
2. Yang M, Baranov E, Jiang P, et al: Whole-body optical imaging of green fluorescent protein-expressing tumors and metastases. *Proc Natl Acad Sci USA* 2000; 97: 1206-1211.
3. Yang M, Baranov E, Moossa AR, Penman S, Hoffman RM: Visualizing gene expression by whole-body fluorescence imaging. *Proc Natl Acad Sci USA* 2000; 97: 12278-12282.