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Indocyanine Green Labeling of Tumors in the Liver Recurring After Radiofrequency Ablation Enables Complete Resection by Fluorescence-guided Surgery

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Abstract. *Background/Aim: Radiofrequency ablation (RFA) is used to treat primary and metastatic tumors in the liver. However, local recurrence after RFA is frequent and subsequent salvage hepatectomy is often ineffective due to difficulty in visualization of tumor margins. Patients and Methods: In the present retrospective clinical trial, seven patients from the Department of General and Gastro-enterological Surgery, Showa University School of Medicine underwent salvage hepatectomy for recurrent hepatocellular carcinoma (HCC) (n=2), colorectal liver metastasis (n=4) and lung-carcinoid liver metastasis (n=1), after RFA, between 2011 and 2020. Tumors were labeled with indocyanine green (ICG) and resected under fluorescence guidance. Resected specimens were evaluated under fluorescence microscopy as well as by standard histopathological techniques. Results: Pathological findings revealed negative tumor margins in all patients after fluorescence-guided surgery. Six of seven resected tumors had a fluorescent rim, including both HCC and liver metastasis. Fluorescence microscopy demonstrated that viable cancer tumor cells were located only on the inside of the fluorescent rim, and no malignant cells were detected within the fluorescent rim surrounding the tumor. Fluorescence microscopy showed that the tumor margin was secured if the fluorescence signal was completely resected. Conclusion: The present results demonstrate that ICG labeling of liver tumors recurring after RFA enabled complete resection under fluorescence guidance. The present study is the first clinical study to demonstrate that tumor types that generally cannot be completely resected with bright light are fully resectable under fluorescence guidance.*

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Key Words: Indocyanine green, ICG, fluorescence-guided surgery, radiofrequency ablation, liver tumors, local recurrence, salvage hepatectomy, tumor margins, R0 resection.

Radiofrequency ablation (RFA) is used to treat small-sized hepatocellular carcinoma (HCC) (1, 2), as it can be safely conducted with minimal morbidity and mortality (3, 4). Currently, depending on the patient's background, RFA is also performed for various liver tumors (5, 6). However, local recurrence is frequent after RFA (5). Reported local recurrence rates have varied from 2.4% to 36% after RFA (7-10). Histologically, residual tumor is frequent in salvage hepatectomy of recurrent HCC after RFA. Due to undetectability of margins of tumors recurring after RFA, major hepatectomy is performed to avoid non-curative resection (11).

In liver surgery, palpation and intraoperative ultrasonography (IOUS) are the primary methods for detecting tumors, confirming tumor location, and determining surgical margins. However, these techniques often do not result in R0 resections. Indocyanine green (ICG) fluorescence imaging is a useful tool in the resection of liver tumors (12-14). ICG is excited by near-infrared light, and emits a fluorescence signal. Previous studies have reported that ICG fluorescence imaging is useful for identifying and securing tumor margins during hepatectomy (15, 16). However, the usefulness of ICG fluorescence imaging

Table I. *Patient characteristics*.

	n=7
Median age (range, y)	72.5 (65-83)
Male/female(n)	4/3
Tumor type	
HCC/CRLM/other	2/4/1
Background liver status	
Normal/chronic hepatitis/cirrhosis	5/2/0
Child–Pugh score, A/B	7/0
ICG R15	9.5 (6-13)
Days between ICG injection and surgery	9.25 (5-21)
Procedure	
Partial resection/subsegmentectomy/ left lateral segmentectomy/ segmentectomy/bisegmentectomy, trisegmentectomy	2/2/0/3/0
Size of tumor (mm) (range)	42.8 (9-130)
Tumor margin	
Positive/Negative	0/7

HCC: Hepatocellular carcinoma; CRLM: colorectal liver metastasis; ICG R15: Indocyanine green retention rate at 15 minutes.

in identifying liver tumors and securing tumor margins for recurrent HCC and liver metastasis after RFA remains unclear. In the present study, we investigated the benefits of ICG fluorescence guidance during salvage hepatectomy for recurrent tumors in the liver after RFA.

Patients and Methods

This study was approved by the Ethics Committee of Showa University School of Medicine and was performed according to the guidelines of the Declaration of Helsinki.

Patients. Between January 2011 and December 2020 at the Department of General and Gastroenterological Surgery of Showa University, seven patients who underwent salvage hepatectomy for recurrent liver tumors after RFA, were recruited for the present retrospective study. Liver tumors included HCC (n=2), colorectal liver metastasis (CRLM) (n=4), and lung-carcinoid liver metastasis (n=1). The patient characteristics are listed in Table I.

Procedure. Preoperatively, 0.5 mg/kg Diagnogreen [indocyanine green (ICG)] (Daiichi Sankyo, Tokyo, Japan) was administered 2-14 days before the surgery. ICG was administered only at these times and not intraoperatively.

Fluorescence microscopy. We previously reported imaging of the ICG fluorescence signal in pathological specimens under fluorescence microscopy (16). The specimens were stained with hematoxylin and eosin. The pathological images were visualized directly under a BZ-X800 fluorescence microscope (Keyence, Osaka, Japan) using an OP-87767 ICG filter (Keyence). The fluorescence width was measured using an analytical application (Keyence).

Table II. *Tumor fluorescence pattern*.

	Partial	Rim	Combined (Partial +Rim)
HCC	1	0	1
CRLM	0	4	0
Other	0	1	0

HCC: Hepatocellular carcinoma; CRLM: colorectal liver metastasis.

Results

Patients and operative characteristics. The patients' characteristics are shown in Table I. The mean diameter of the tumors was 42.8 mm (range=9-130). Open hepatectomies were performed. Histopathological analysis, with hematoxylin-eosin-stained slides of paraffin-embedded tumor tissue, demonstrated negative tumor margins in all patients. Thus fluorescence-guided surgery (FGS) for recurring tumors after RFA labeled with ICG was highly effective.

Fluorescence microscopic pathological findings. Table II shows the tumor fluorescence patterns classified as previously reported (16). Among the specimens, two cases were well-differentiated HCC. The fluorescence patterns of HCC were partial fluorescence (n=1) (Figure 1) and combined partial + rim fluorescence (n=1). The pattern of all liver metastases was rim fluorescence (*i.e.*, a fluorescence signal only surrounding the tumor) (Figure 2). In the rim-fluorescence cases of both HCC and liver metastasis, viable cancer cells were not located within the fluorescent rim and only on its inside.

Discussion

Since the first report on RFA of liver tumors in 1992 (17), it has become widely used as a primary treatment for liver tumors. Some reports suggested that RFA for HCC offers beneficial short-term outcomes and is comparable to hepatectomy for long-term outcomes (3, 4). However, local recurrence after RFA has been a major problem. Salvage hepatectomy for recurrent HCC or liver metastasis after RFA is technically difficult, perioperatively long, and postoperatively leads to complications (18-21), due to difficulty in visualizing tumor margins. Yamagishi *et al*. (11) reported that residual tumor was more frequently observed in salvage hepatectomy after RFA. The incidence of vessel involvement was significantly higher in local-tumor recurrence after RFA (11, 18). Locally-recurrent tumor may develop to a more advanced stage with unclear margins after RFA. Additionally, detection of recurrent tumors through ultrasonography (US) is difficult because of the echogenic

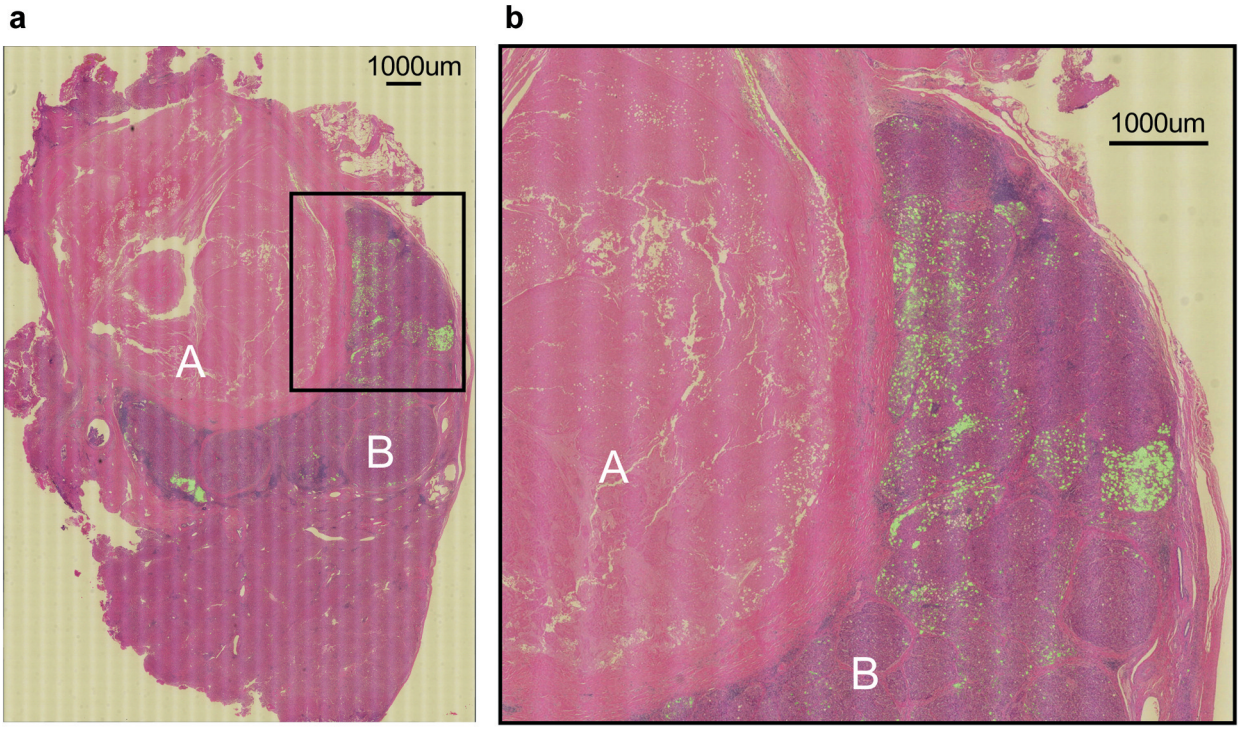

Figure 1. *Microscopic fluorescence findings of resected tumor specimen after fluorescence-guided surgery with indocyanine-green labeling of local recurrence of hepatocellular carcinoma (HCC) after radiofrequency ablation (RFA). (a) Fluorescence microscopy image (original magnification ×1); partial fluorescence type. (b) Magnified image within the box in (a) showing secured tumor margin with no fluorescence in the noncancerous liver tissue. Inlet: (A) Post-RFA lesion, (B) local recurrence of HCC.*

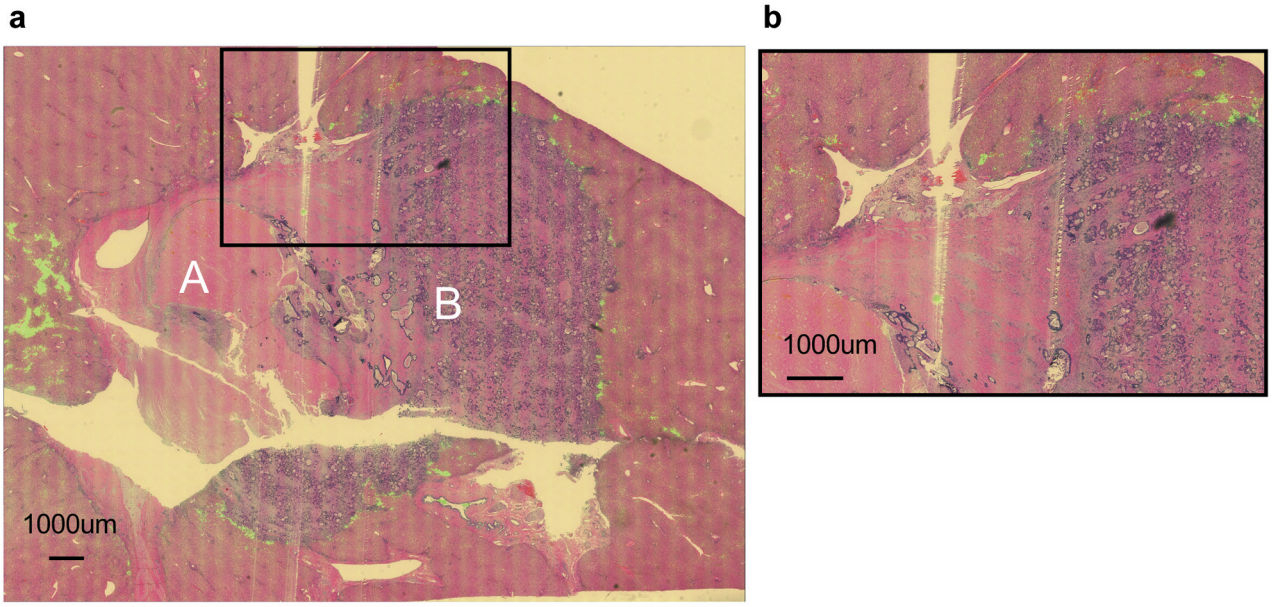

Figure 2. *Microscopic fluorescence findings of resected tumor specimen after fluorescence-guided surgery with indocyanine-green labeling of local recurrence of colorectal liver metastasis (CRLM) after radiofrequency ablation (RFA). (a) Fluorescence microscopy images (original magnification ×1); rim fluorescence type. (b) Magnified image within the box in (a) showing secured tumor margin with no fluorescence in the noncancerous liver tissue. Inlet: (A) Post-RFA lesion, (B) local recurrence of CRLM.*

response after RFA. Post-RFA lesions were visualized as hyperechoic regions by US. Even lesions that are clearly identified by contrast CT and MRI may not be clearly identified through US because of background hepatic conditions or artifacts from previous cauterization (22). Therefore, preventing local recurrence after RFA requires reliable tumor-visualization methods.

Currently, ICG is used in fluorescence-guided surgery for tumors in various organs (23-27). It is also used for highly-sensitive identification of liver tumors (28), mapping for liver segmentation (12), biliary tract surgery (14), and bile leakage (29). The present results demonstrate that ICG enables effective fluorescence-guided surgery of liver tumors that recur after RFA that are often incompletely resected under bright light which does not allow tumor margins to be visualized. However, ICG fluorescence has some disadvantages, such as difficulty in detecting deeply-located tumors and a high rate (approximately 40%) of false positives due to ICG uptake by non-malignant tissues.

The local-recurrence frequency appears to be significantly higher after RFA of tumors located on the liver surface, than in tumors located deep within the liver (30-32). ICG fluorescence is suitable for complete tumor visualization on the liver surface. The goal of surgical resection in cancer is to obtain an R0 status, which indicates negative surgical margins. In the present study, the tumor margin was secured by fluorescence-guided surgery in all cases. This is the first study to investigate the pathological validity of using ICG fluorescence as an indicator of surgical-margin status during hepatectomy of recurrent and metastatic liver tumors after RFA. For both HCC and liver metastasis, viable cancer cells were located only on the inside of the fluorescent rim of the resected tumor, and no malignant cells were detected in the fluorescent rim surrounding the tumor, indicating FGS resulted in secure tumor margins (Figure 1 and Figure 2).

Our laboratory pioneered fluorescence-guided surgery of liver metastasis in orthotopic mouse models, providing proof-of-concept for the present study (33-38).

Conclusion

The present study is the first to demonstrate that fluorescence-guided surgery (FGS) can result in R0 resection of a tumor type that could only rarely be completely resected under bright light. The present study thus demonstrates the important clinical potential of FGS for difficult-to-resect tumors.

Conflicts of Interest

There are no financial or other interests about the submitted manuscript that might be construed as conflicts of interest.

Authors' Contributions

Y.T. and T.A. designed experiments. Y.T. performed experiments, Y.T. analyzed data and wrote the paper. T. H., T. K., T. K., K. M., K. Y., K. N., T. H., Y.W., H. S., K. T., T. Y., K. S., A. F., Y. E., and R.M.H gave technical support and conceptual advice. RMH revised the manuscript.

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