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Intraoperative Detection of Liver Tumors Aided by Fluorescence Goggle System and Multimodal Imaging

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Abstract

Real-time image guidance in the operating room is needed to improve instantaneous surgical decisions. Toward this goal, we utilized a new fluorescence goggle system and a near-infrared fluorescent dye approved for human use, indocyanine green, to demonstrate the feasibility of detecting liver tumors intraoperatively. The fluorescence goggle provided successful imaging of multifocal breast cancer metastases in mouse liver. Diffused tumor deposits as small as 0.8 mm in diameter were detected, which were not obvious without the fluorescence goggle. A combination of surface-weighted fluorescence imaging and deep tissue-sensitive ultrasound imaging allowed comprehensive image guidance with the fluorescence goggle system for tumor resection in a rabbit VX2 liver metastasis model. This multimodal detection and guided surgical intervention strategy using ultrasonic imaging and real-time intraoperative fluorescence guidance is a promising and innovative technology platform for improving surgical outcome of human patients with primary or metastatic liver cancer.

Clinical management of liver tumors is a challenging medical problem. There are two types of liver tumors that cause significant morbidity and mortality today: primary tumors and the liver metastases. The most prevalent form of primary liver tumors is hepatocellular carcinoma (HCC), which alone leads to 600,000 mortalities annually.[1, 2] In addition to HCC, metastatic spread of breast, melanoma, and colon cancer to the liver is common.[3–5] Unfortunately, the current standard of care for liver tumors is not satisfactory. As liver tumors do not respond well to chemo-, radio- or hormone therapy, curative options are primarily limited to surgical resection, liver transplantation and, to some extent, radiofrequency ablation (RFA).[1, 2] Oftentimes, liver transplantation is not feasible because of the scarcity of donor liver, a situation that is particularly complicated in metastatic disease. Compared to RFA, which is mainly effective for small tumors, surgical resections show significantly improved survival benefits and lower complications for patients with early stage liver tumors,[6] rendering it the primary method of intervention. Although the resections of liver tumors are routinely performed in major hospitals, it is difficult for surgeons to completely remove all the lesions, including small tumor deposits that can become aggressive after surgery. Thus, the surgical outcomes are usually poor, leading to cancer relapse within a year of initial surgery.

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Despite the sensitivity of MRI and CT in detecting liver tumors, the large deformation of liver during surgery makes it difficult to correlate the anatomical landscape with the pre-operative images. Surgeons rely on naked eye observation, palpation and intraoperative ultrasound (IUS) to differentiate tumors from healthy liver tissues.[1, 2] Naked eye observation and palpation are not sufficiently sensitive to visualize small tumors.[1] As the primary clinical intra-operative imaging modality, ultrasound provides moderate sensitivity and contrast, but suffers from small field of view and poor contrast close to tissue surfaces.[7, 8] The sensitivity of IUS is poor for isoechoic, small and surface-located tumors. In addition, the need for physical contact of ultrasound probe with tissue imposes a discontinue workflow in the operating room or the need for additional clinicians to operate the instrument.

In the past decade, near infrared (NIR) fluorescence imaging has been intensively investigated as a new intra-operative imaging modality because of the low autofluorescence and enhanced tissue penetration of light in this spectral region.[9–13] However, most of the reported intra-operative imaging systems use computer monitors to display imaging results, which may potentially distract the surgeon and compromise surgeon's hand-eye coordination. To overcome this challenge, we previously developed a compact wearable fluorescence imaging and display system, the fluorescence goggle.[14] We demonstrated that the goggle facilitated primary breast tumor resection and sentinel lymph node biopsy in animal models.[14]

It was reported recently that ICG could detect primary liver tumors including HCCs for guided operation.[15, 16] The ability to image primary liver tumors is important, particularly in Asia and Africa where HCC is very prevalent. In contrast, the occurrence of liver metastases is more prevalent than that of primary tumors in North America. Therefore, a combination of fluorescence and ultrasound imaging would provide a rapid intraoperative assessment of primary and metastatic tumors in the liver through complementary surface and subsurface-weighted images in real time.

In the current study, we investigated the feasibility of intra-operative detection of liver tumors aided by the fluorescence goggle, a problem of clinical significance worldwide that needs investigation.[3–5] To facilitate clinical use in clinical trials, we employed indocyanine green (ICG), which is approved for human use worldwide, as the contrast agent.

We developed a mouse model of breast cancer metastasis to the liver by direct implantation of breast cancer cells (4T1-luc) in the liver (Supplementary Information). All animal procedures were conducted in compliance with Washington University Animal Studies Committee's requirements for the care and use of animals in research. The 4T1-luc cells express luciferase, which interacts with its exogenous substrate (luciferin) to produce bioluminescence used for imaging the distribution of tumors *in vivo*. With emission peak at 560 nm, the bioluminescence does not interfere with the NIR fluorescence of ICG, centered at 830 nm. Since bioluminescence positively correlates with the tumor size, this technique is a convenient approach to locate primary tumors and monitor their growth in small animals. Thus, co-localization of bioluminescence and NIR fluorescence of ICG provides *in vivo* validation of tumor detection with the clinically translatable NIR imaging using the fluorescence goggle system. ICG was injected intravenously (i.v.) at a dose of 0.5 mg/kg body weight, which is the approved dose for liver function tests in humans. Therefore, a single dose could enable both liver function test and intraoperative imaging. The optimal imaging time was also studied. The mice were divided into two groups with imaging at 48 h (Group 1, n=6) and 24 h (Group 2, n=5) post-injection.

At 48 h post-injection, image-guided liver surgery was performed on Group 1 mice. Prior to surgery, the presence of liver metastases was confirmed by bioluminescence imaging (Supplementary Figure 1). Intraoperatively, the fluorescence goggle clearly detected the liver metastases in 5 out of 6 mice, which were not grossly evident to naked eye observation or by palpation (Figure 1A–C). Under fluorescence guidance, liver resections were readily performed. The fluorescence signal in the surgical bed decreased to background levels seen in the healthy liver at the completion of the surgery. This indicates that the goggle facilitated an efficient removal of positive nodules from the liver. The liver tumors were subsequently examined *ex vivo* to confirm fluorescence signal (Figure 1D–F).

In addition to single tumor detection, the goggle also detected diffused satellite lesions that would have been otherwise missed (Figure 2A–C). Some of these lesions were as small as 0.8 mm in diameter. In accordance with our observations in vivo, small satellite metastases were clearly delineated by the fluorescence goggle when examined ex vivo (Figure 2D–F). The average tumor-to-liver contrast for Group 1 mice was (2.79±0.72:mean±std). The histological findings confirmed selective fluorescence of tumor tissues as observed *in vivo* (Supplementary Figure 1).

In contrast, fluorescence imaging was less effective for Group 2 mice, which were operated 24 h after injection of ICG. Fluorescence imaging only detected tumors in 2 out of 5 mice. In these 2 mice, the liver tumors as well as most part of intestines showed high fluorescence signal (Supplementary Figure 2). The average tumor-to-liver contrast in Group 2 mice is (1.54±0.74: mean±std). The results indicate that the tumor-to-liver contrast of Group 1 was significantly higher than that of Group 2 ($p < 0.05$). ICG is known to accumulate in liver tumors primarily through enhanced permeability retention (EPR) and reduced local hepatobilliary clearance function [15, 16]. Thus, a possible explanation for the disparity observed between the Group 1 (48 h) and the Group 2 (24 h) mice is that at 24 h, the ICG was still actively undergoing hepatobilliary clearance, leading to higher background fluorescence in the liver and the intestines. Conversely, at 48 h post-injection, ICG in the liver had cleared from the normal liver, except in the liver tumors, where it is retained by EPR effect, leading to better contrast.

Furthermore, we explored the feasibility of using the fluorescence goggle to guide liver surgery in larger animals as a more realistic model of human surgery. A rabbit liver cancer model was used because of its closer physiological resemblance to humans than rodents. Briefly, a 2 mm midline incision was made through which VX2 tumor tissues were implanted into the liver using a Trocar needle under ultrasound guidance similar to literature method (Supplementary Figure 3).[17] Compared to traditional tumor implantation method performed under laparotomy, this technique is much less invasive and less likely to cause death or major side effects.

At 2 weeks after implantation of liver tumor, we conducted the liver resection aided by ultrasound and the fluorescence goggle imaging, followed by histologic validation of the excised tumor tissue. The timing and dosing of ICG injection was determined from the data obtained from our rodent models. ICG was administered intravenously 48 h prior to surgery at a concentration of 0.5 mg/kg body weight. The presence of tumors was first confirmed by ultrasound imaging (Figure 3A). Liver tumors were subsequently imaged intraoperatively using the fluorescence goggle system in real time. The tumors showed higher fluorescence signal than surrounding tissues (Figure 3B). With image-guidance from the fluorescence goggle system, the extension of the tumor was visualized. Clean surgical margin was obtained without removing excessive normal liver tissue, as validated by histopathology. Histology also confirmed that the putative tumors identified *in vivo* were cancerous (Figure 3C). Our observations in the rabbit model agreed well with those of the mouse model. This

study demonstrated the feasibility of integrating goggle-aided fluorescence imaging into the operating room workflow and deploying multiple modalities for intraoperative liver cancer detection.

The above promising results also identified some limitations of the current approach. First, we used ICG to guide surgery and provide feasibility of translating the current system into the clinic. However, ICG is not highly tumor-selective and has difficulty detecting neoplastic lesions with high sensitivity and specificity.[18] This shortcoming accentuates the need for cancer-selective molecular imaging agents approved for use in humans to provide higher tumor-to-normal tissue contrast than ICG. Second, the current study clearly demonstrates the feasibility of detecting liver tumors aided by the fluorescence goggle and multimodal imaging. However, future studies will utilize spontaneous liver tumor models with larger number of animals to establish the sensitivity and specificity of the method with a more realistic clinically relevant model.

In summary, we have demonstrated the feasibility of guiding the surgical resection of primary and metastatic liver tumors using the fluorescence goggle system in conjunction with ICG both in a rodent model and a rabbit model. The goggle system was easily adaptable to standard operating room (OR) settings for liver resection. Small lesions and satellite lesions were clearly detected. Imaging at 48 h post injection provided a better contrast than the 24 h in the rodent model. The feasibility of multimodal imaging with both ultrasound and goggle-aided fluorescence imaging was also demonstrated. With the accurate intraoperative imaging of liver metastases, more efficient tumor resection, with attendant negligible residual tumors, could significantly improve patient outcomes, potentially resulting in a more curative than a palliative outcome. The fluorescence goggle system has shown great potential in complementing existing clinical modalities and guiding liver surgeries.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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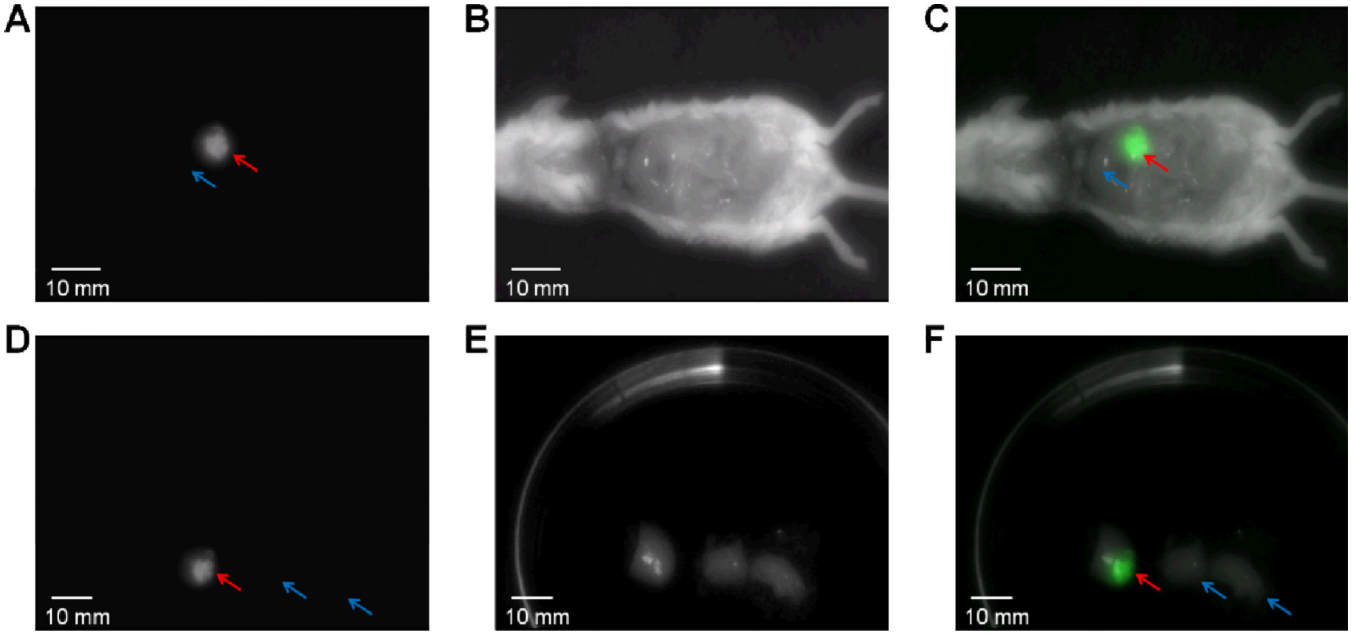

Figure 1.

Detection of breast cancer liver metastases with fluorescence goggle and ICG in Group 1 mice. Intraoperative (A) NIR fluorescence image, (B) reflectance image and (C) merged image of (A) and (B) of a mouse 48 h post-injection of ICG are shown. Fluorescence goggle readily detects the metastases in the liver intraoperatively. Ex vivo (D) NIR fluorescence image, (E) reflectance image and (F) merged image of (D) and (E). NIR fluorescence is pseudo-colored in green in (C) and (F). Red arrows indicate the metastasis, and blue arrows indicate the liver.

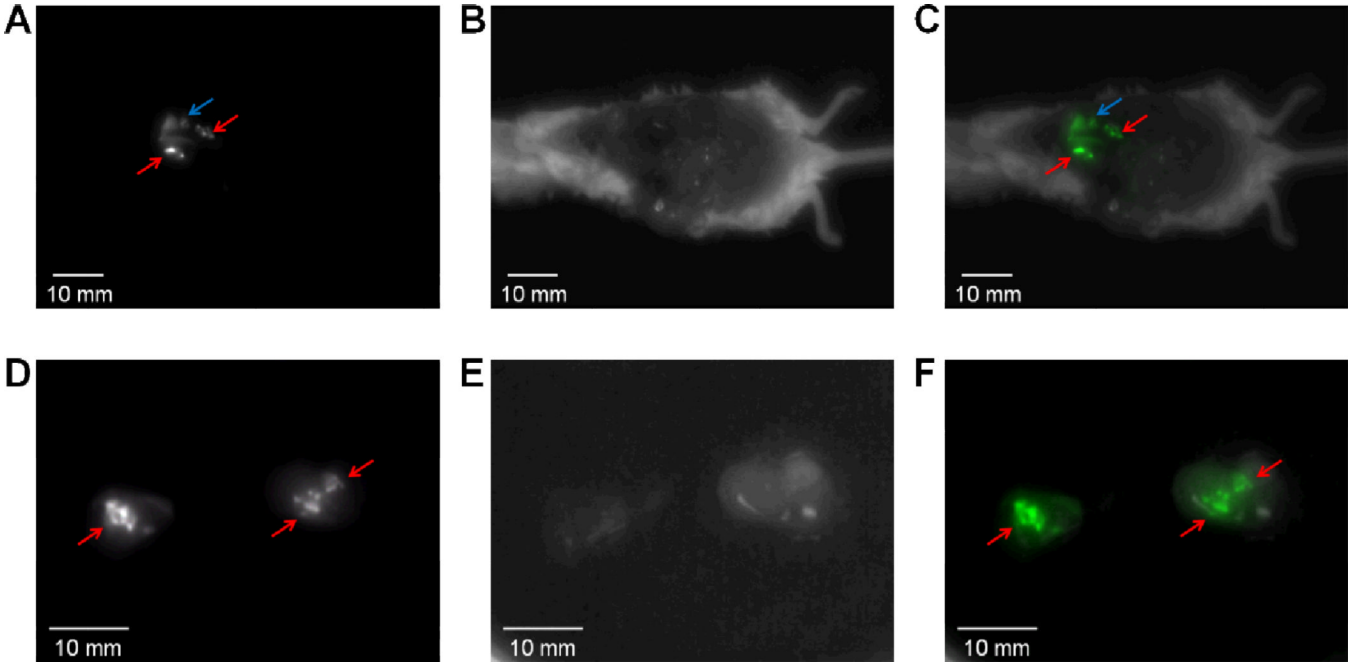

Figure 2.

Detection of diffuse satellite lesions with fluorescence goggle and ICG in Group 1 mice. Intraoperative (A) NIR fluorescence image, (B) reflectance image and (C) merged image of (A) and (B) of a mouse 48 h post-injection of ICG are shown. Fluorescence goggle detects scattered metastases and small tumor deposits in the liver intraoperatively, which are not obvious to naked eye. Ex vivo (D) NIR fluorescence image, (E) reflectance image and (F) merged image of (D) and (E) are shown. NIR fluorescence is pseudo-colored in green in panels (C) and (F). Red arrows indicate the metastasis, and blue arrows indicate the liver.

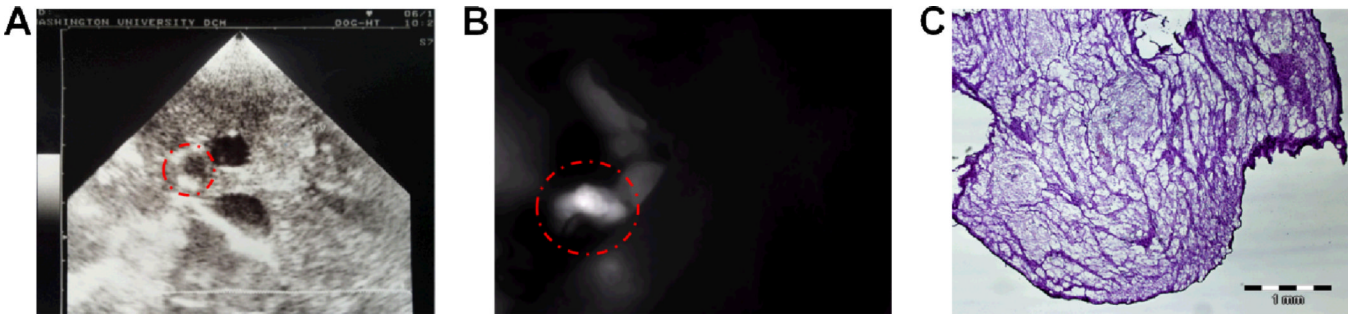

Figure 3.
Multimodal detection of liver tumors in rabbits. (A) Ultrasound image; (B) intraoperative fluorescence image; (C) H&E image. Red cycles indicate the tumors. The combination of ultrasound, goggle-aided NIR fluorescence and histology successfullydetected and identified the liver tumors.