Unexpected detection of a submillimeter early hepatocellular carcinoma focus by intraoperative near-infrared fluorescence imaging—a case report

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Background: Prognosis of hepatocellular carcinoma (HCC) is closely related to residual tumor cells and tissues after tumor resection. Thus, close monitoring to ensure complete removal of residual tumor is fundamental. In this regard, intraoperative near-infrared fluorescence (NIRF) imaging has been of great assistance to surgeons for precision cancer surgeries. However, up to now, the identification of tiny lesions has not been reported. Herein, we report our findings on the case of an ultra-small HCC focus of about 430 μm that was successfully detected using NIRF during real-time monitored liver cancer surgery. The patient had a background of hepatitis B cirrhosis, which is the most phenomenon in China. Surgeons usually unable to distinguish sclerotic nodules from small tumor tissue with the naked eyes.

Case Description: A 55-year-old man with chronic hepatitis B infection was preoperatively diagnosed with a space-occupying liver lesion. A fluorescence signal was detected on the surface of the liver through the NIRF imaging system which had not been found by preoperative computed tomography (CT) and ultrasound examination. We subsequently tested the residual liver surface and observed a high signal point, less than 1 mm in the right anterior lobe of the liver. Histopathological examination revealed that the tiny fluorescent spot belong to an early HCC focus.

Conclusions: Based on these results, we think indocyanine green (ICG)-NIRF imaging may be used as a routine intraoperative detection method for liver cancer surgery in order to remove any residual tumor cells and tissue, hence minimizing further risk of remnant tumor regrowth.

Keywords: Indocyanine green (ICG); tiny lesion; micrometastasis; hepatocellular carcinoma (HCC); case report

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Introduction

Subsequent to the identification of sentinel lymph nodes by Kitai *et al.* (1) using indocyanine green (ICG), intraoperative near-infrared fluorescence (NIRF) imaging technology has emerged as a research focus for precise image-guided surgeries, enhancing our understanding of several domains of surgical oncology over the past decade. Numerous reported clinical cases, including detection of non-small cell lung cancers (2), thymic malignancies, neuroendocrine lung malignancies, pleural mesothelioma, thoracic metastases (3),

and oral squamous cell carcinoma (4), have demonstrated that NIRF provides assistance to surgeons in three major ways: primary tumor localization, identification of positive surgical margins, and detection of remote satellite cancerous lesions.

Ultra-small residual tumors play a major role in determining patients' postoperative prognosis and survival. Once the dysplastic lesion is larger than 2 cm, the chance of cure decreases significantly (5). Subcentimeter tumors have been previously identified in an ovarian cancer animal xenograft model (6) and in phase I clinical trials using second window ICG in thoracic cancer (7). To the best of our knowledge, herein we have reported for the first time the case of an early hepatocellular carcinoma (HCC) focus of about 430 μm that was visualized during real-time image-guided surgery. Regardless of using electromagnetic, ultrasonic, or fluorescence techniques, this submillimeter lesion was detected on the liver of a 55-year-old man diagnosed with a space-occupying neoplasm of the liver. The case is a part of a clinical study assessing the role of NIR imaging in guiding small-lesion detection during surgery. To date, the study has involved 13 patients (age range, 45–70 years) diagnosed with hepatocarcinoma.

HCC is the third leading cause of cancer-related death in the world (8). For patients with early-stage liver cancer, surgery is still the main treatment method. However, the recurrence of liver cancer after resection remains a major concern, with a 5-year recurrence rate greater than 70% (9), leading to poor prognosis. Even patients with small HCC (<3 cm) who undergo surgery have a 5-year survival rate of only 47–53% (10-12). Lack of effective intraoperative diagnosis is one of the key factors leading to residual tumor cells and postoperative recurrence. The NIRF imaging system has been widely used for the removal of sentinel lymph nodes during breast cancer surgery (13) and is currently used in the localization of tumors during liver cancer surgery (14,15). However, to the best of our knowledge, the role of the NIRF imaging system in detecting tiny lesions in normal liver tissue has not been reported elsewhere.

In this study, after using the NIRF imaging system to locate the primary tumor, we further examined the surrounding normal liver tissue and unexpectedly identified a tiny bright spot with a diameter of about 1 mm, which was excised under real-time NIRF imaging system guidance. Postoperative pathology confirmed that the tiny spot corresponded to an early HCC focus with a diameter of about 430 μm. These precancerous cells were not detected during preoperative imaging assessment. This finding confirmed the complementary role of NIRF imaging during liver cancer resection. Furthermore, NIRF could be used to perform intraoperative observation of the entire liver, identify tiny lesions, and reduce the postoperative recurrence rate. We present the following article in accordance with the CARE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2338/rc).

Case presentation

The 55-year-old man, was feeling pain and discomfort in the liver area for several months and had no obvious related medical history. The patient was infected by chronic hepatitis B virus for decades and has not received regular antiviral therapy. Through pre-hospital ultrasound and CT scans, he was preoperatively diagnosed with a space-occupying liver lesion. Before the operation, we carried out the regular antiviral treatment and liver protection drug treatment for the patient for nearly a week. Pre-surgery laboratory test results showed that at 77.9 ng/mL, the concentration of tumor marker alpha-fetoprotein (AFP) was higher than normal limits (*Table 1*). Liver function indexes were in normal range, whereas ICG retention at 15 minutes (ICG-R15) was 18.9%, which was beyond 15%, indicating that liver metabolism was impaired. Therefore, our assessment was that this patient could only undergo hepatic lobectomy with preoperative tumor localization by computed tomography (CT) (*Figure 1*). We used an ICG-NIRF imaging system endowed with a qualitative detection limit of 0.488 nM and a quantitative detection limit of 3.91 nM (*Figure 2*) to observe the tumor and attempt to identify any other tiny tumors in the residual liver. The patient, who had not previously undergone a surgery of this type, was first injected with ICG (0.577 mg/kg ICG) intravenously. However, due to the patient's impaired metabolism, the surgery was rescheduled for 4 days after the ICG injection in order to obtain an optimal tumor-to-background ratio. The fluorescence signal that emitted from the liver surface could be detected through the NIRF system and could also be clearly recognized when the specimen was analyzed *in vitro* (*Figure 3*). After the clear lesion was removed, we observed no residual lesions during the operation. It may due to the shape of the liver cirrhosis nodules affected our judgment. We subsequently tested the residual liver surface with the NIRF system and observed a high signal point of about 1 mm in the right anterior lobe of the liver, which

Table 1 List of the patient's laboratory test results before surgery

Laboratory tests	Results
ALT	42.1 U/L
AST	27.9 U/L
GGT	27.2 U/L
AKP	81.7 U/L
TBL	16.6 μmol/L
DBL	6.2 μmol/L
ALB	42.2 g/L
AFP	77.9 ng/mL
CEA	2.42 ng/mL
CA125	10.20 ng/mL
CA19-9	24.14 ng/mL
CA72-4	0.9 ng/mL
CA242	2.93 ng/mL
ICG_{R15}	18.9

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; AKP, alkaline phosphatase; TBL, total bilirubin; DBL, direct bilirubin; ALB, albumin; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4; CA242, carbohydrate antigen 242; ICG_{R15}, indocyanine green retention rate at 15 minutes.

showed no differences from normal liver through naked-eye observation and palpation. In our previous case, no result showed the NIRF system can detect lesions smaller than 1cm, and due to the limitations of fluorescence technology, it is impossible to determine whether it was an inflammatory tissue, but the high intensity of fluorescence raised our suspicions. After excision, the specimen also sustainably expressed high fluorescence (*Figure 4*). Finally the histopathological examination revealed that this tiny fluorescent spot belong to a single focus of an early HCC (*Figure 5*). On the histopathological images, steatosis and Mallory-Denk bodies were identified. Meanwhile, multinucleation and nuclear atypia were also detected, displaying nuclear overlapping, size enlargement, and nuclear membrane distortion. The surgery lasted 235 minutes and intraoperative bleeding was 200 mL. The patient had good compliance and no discomfort was reported following the surgery. The follow-up of the patient was done through periodic CT scans and AFP-level testing to further assess the HCC prognosis. The patient's liver function and AFP were close to normal before discharge. So far, no tumor recurrence has been found in the follow-up.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Declaration of Helsinki (as revised in 2013). Written

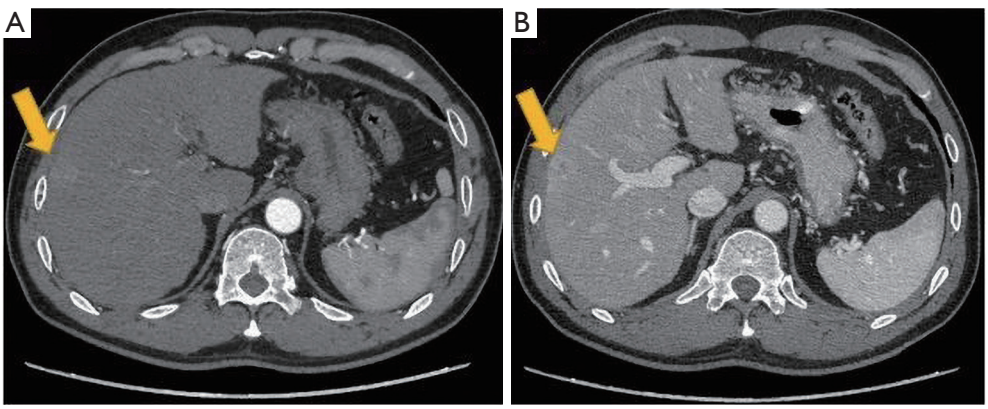

Figure 1 Enhanced CT showed a subhepatic space-occupying lesion in the right lobe of the liver and no obvious abnormalities were found. (A) Early arterial phase; (B) delayed phase. The yellow arrows indicated the space-occupying lesion. CT, computed tomography.

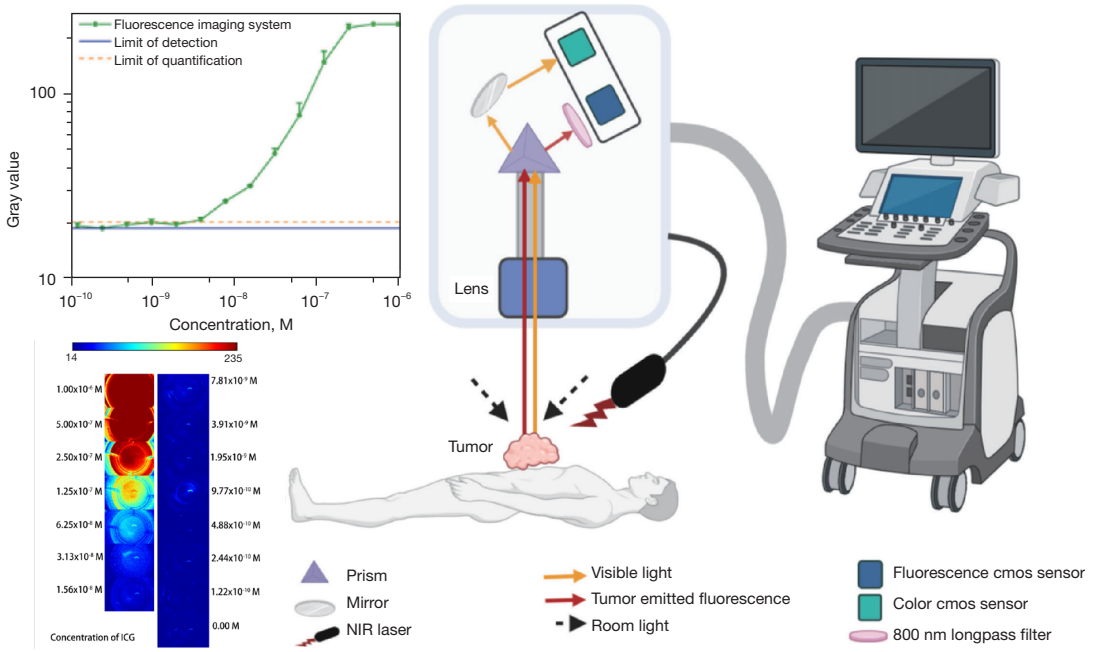

Figure 2 Schematic diagram of NIR-real-time intraoperative guided liver resection using ICG. On the left are shown the results of the preoperative *in vitro* imaging of ICG solutions in 4% m/v BSA/PBS at different concentrations, the fluorescence signals of each well were quantified and the gray values were plotted against the concentrations, the qualitative detection limit was 0.488 nM, and the quantitative detection limit was 3.91 nM. NIR, near-infrared; ICG, indocyanine green; BSA, bovine serum albumin; PBS, phosphate-buffered saline.

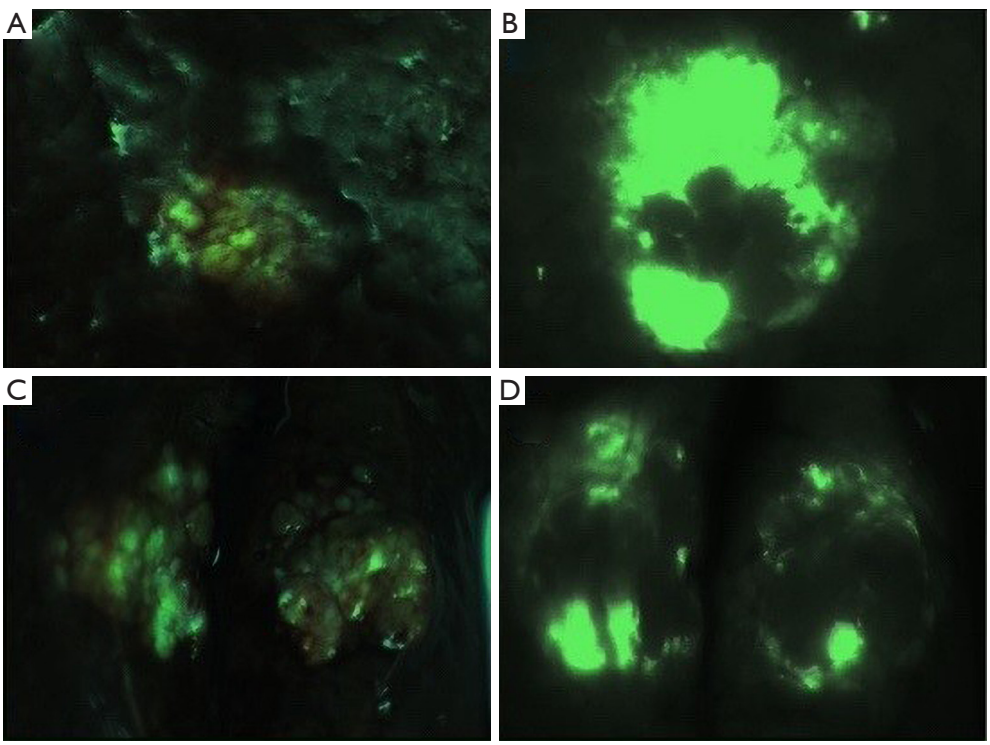

Figure 3 Findings using NIRF system. (A,B) Recurrent lesions were located on the surface of the liver; (C,D) *in vitro* imaging of dissected tumors. NIRF, near-infrared fluorescence.

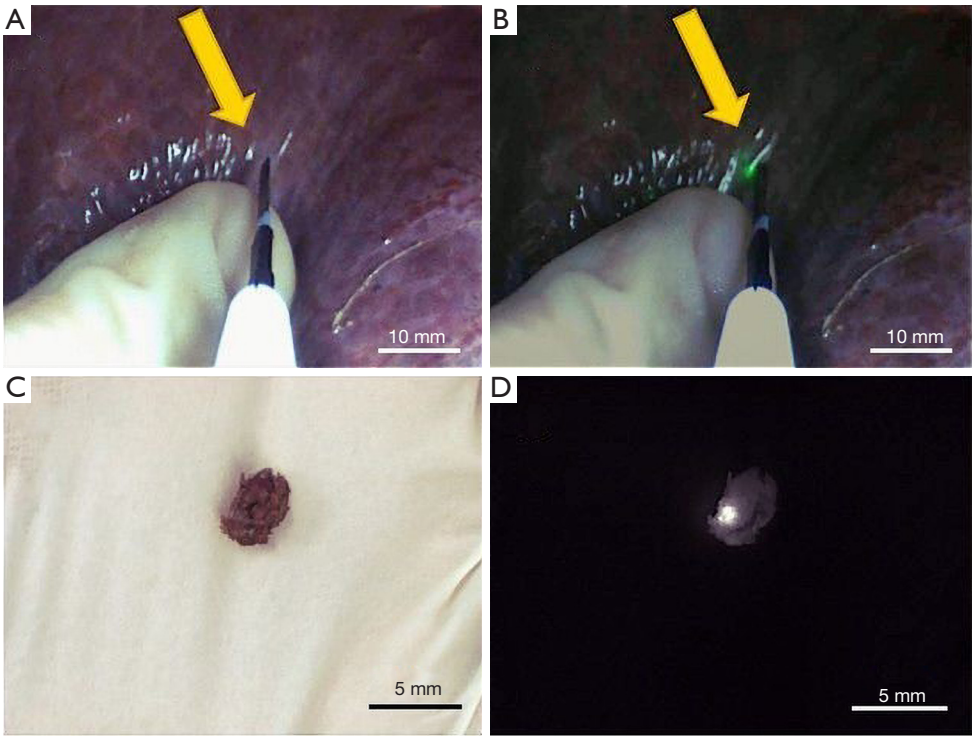

Figure 4 Detection of tiny lesions using NIRF system. (A) Visual inspection of the lesion during the surgery; (B) system fluorescence detection of the lesion; (C) lesion resection; (D) *in vitro* fluorescence of the tiny lesion. The yellow arrows indicated the location of the submillimeter early tumor *in vivo*. NIRF, near-infrared fluorescence.

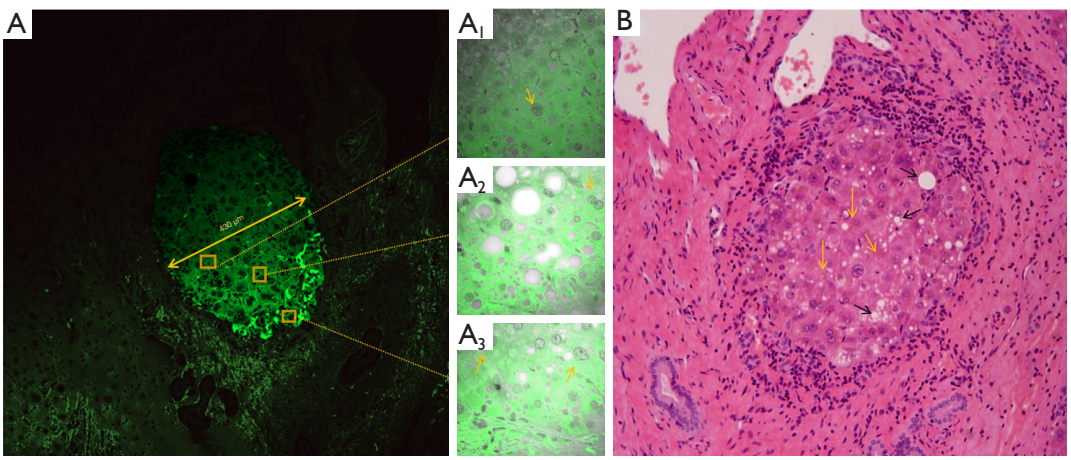

Figure 5 Pathological and fluorescence images of the resected specimen. (A) Confocal fluorescence image of the focus (×20). The yellow arrow indicated the short axial length of the submillimeter lesion. A_1-A_3 are confocal bright field and fluorescence overlay images, showing nuclear pleomorphism with overlapping, mitosis, and nuclear size enlargement, and nuclear membrane distortion (×40). (B) H&E stain image of the focus showing steatosis (black arrows) and presence of Mallory-Denk bodies (yellow arrows) (×20). At high magnification, hyperchromasia and multinucleation were also detected. H&E, hematoxylin and eosin.

informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

Complete resection of a tumor depends on its precise location and the identification of small lesions by preoperative imaging. Subjective judgment of the surgeon used to be the main method during surgery to ensure a negative margin and the removal of small lesions. Unfortunately, about 40% patients have residual tumor cells during surgery (16). It has been shown that imaging tests before surgery, such as CT and MRI, were not suitable for the detection of micrometastases and nodules (<5 mm) (17) due to the infiltration of cancer tissues, lack of differentiation, nerve involvement, and complex veins. The tube system and others are based on the surgeon's inspection and palpation, leading to a significant tumor residual rate (exceeding 50%) (18). Through the enhanced permeability and retention (EPR) effect of tumor tissue, fluorescent dyes can accumulate in tumor tissue, and the NIRF imaging system could provide high tumor-to-background signal ratio using large amounts of ICG, thereby helping surgeons to effectively localize tumors (19,20). However, despite recent advances in imaging modalities, about 3–17% of HCCs can only be detected by microscopic examination (21,22). Since it has been reported that tiny lesions lack effective blood vessels and EPR effect, the principle of fluorescence imaging of small lesions needs further investigation.

In this study, we discovered that the NIRF imaging system could detect a tiny early HCC focus, which may have been related to the ultra-high sensitivity of the detection instrument, the dose of the injected fluorescent dye, and the injection time. Even though the sampling size was small and the mechanism requires further research, this technology could effectively improve tumor clearance rate and reduce tumor recurrence, and thus should be implemented as a routine intraoperative detection method. The use of ICG in several NIR-guided oncological surgeries has shown promising results. Nevertheless, further comprehensive research and clinical experiments are required in order to understand the overall effects of ICG.

Conclusions

Our results strongly suggested that the ICG-NIRF imaging system should be used as a routine intraoperative detection method for liver cancer surgery in order to remove any residual tumor cells and tissue, hence minimizing further risk of remnant tumor regrowth.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2338/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2338/coif). HC reports that he is the owner of Nanjing Nuoyuan Medical Devices Co., Ltd., which produced the ICG-NIRF imaging system. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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