Scientific Basis and Clinical Application of ICG Fluorescence Imaging: Hepatobiliary Cancer

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Abstract: Despite recent advances in imaging modalities, the intraoperative diagnosis of small liver cancers remains unsatisfactory. Although fluorescent imaging using indocyanine green (ICG) has just been applied to hepatobiliary surgeries, this technique has the potential to delineate small liver cancers during surgery, through allowing visualization of the disordered biliary excretion of ICG in the hepatocellular carcinoma (HCC) tissues and non-cancerous liver tissues surrounding metastasis of colorectal cancer (CRC). In this technique, ICG is administered intravenously for routine liver function testing before surgery, at the dose of 0.5 mg per kg body weight. The liver surfaces prior to resection, and the cut surfaces of the resected specimen, are examined by the fluorescent imaging system. In our previous series, ICG-fluorescent imaging prior to resection delineated more than 90% of liver cancers that were located within 10 mm of the liver surface. On examination of the cut surfaces of the resected specimens, this technique identified all of the microscopically confirmed HCCs and metastases of CRC. Furthermore, ICG-fluorescent imaging was useful to detect small HCCs that were not evident grossly unless visualized by this technique, as reported by Gotoh *et al*. These results suggest that ICG-fluorescent imaging enables the highly sensitive identification of small liver cancers in real time during liver resection and the subsequent macroscopic examination, enhancing the accuracy of surgery and operative cancer staging.

Keywords: ICG fluorescence imaging, cancer detection, hepatocellular carcinoma, liver metastasis of colorectal cancer, disordered bile excretion.

INTRODUCTION

Although liver resection has been established as an effective therapeutic choice for hepatocellular carcinoma (HCC) and metastasis of colorectal carcinoma (CRC), the intraoperative diagnosis of liver cancers remains unsatisfactory: despite recent advances in imaging modalities, 3 - 17% of liver cancers can only be detected by microscopic examination [1-5]. In particular, we have often encountered difficulties in identifying small HCC with indistinct margins (early HCC) [6-9]. Such liver cancers that cannot be identified grossly may be overlooked at the time of operative or even pathological diagnosis, since thorough microscopic examination of the entire volume of the resected specimens is not practically feasible. Novel intraoperative imaging techniques are needed to further enhance the accuracy of liver resection and the subsequent pathological diagnosis.

Recently, fluorescent imaging techniques using indocyanine green (ICG) have been applied to cardiac surgery [10, 11] microvascular surgery [12, 13] and surgeries for breast [14, 15] and gastrointestinal cancers [16-18]. These techniques are based on the principle that ICG binds to plasma proteins and protein-bound ICG emits light with a peak wavelength of around 830 nm when illuminated with near-infrared light [19]. We have developed a fluorescent imaging technique for intraoperative identification of liver cancers, utilizing the property of intravenously-injected ICG being exclusively excreted into the bile; we hypothesized that fluorescent imaging would enable real-time identification of liver cancers through allowing visualization of the disordered biliary excretion of ICG in HCCs and in the non-cancerous liver tissue surrounding metastases of CRC [20]. In this article, we review the procedure and basic findings of fluorescent imaging technique to identify liver cancers during surgery.

ICG-FLUORESCENT IMAGING TECHNIQUES FOR IDENTIFICATION OF LIVER CANCERS

Administration of ICG

As the source of fluorescence, we can utilize ICG (Diagnogreen, Daiichi Sankyo, Tokyo, Japan), which was injected intravenously 1 - 14 days prior to the surgery at the dose of 0.5 mg per kg body weight as part of routine liver function testing to determine the operative indications and procedures [21, 22].

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Fluorescent Imaging System

We use the fluorescent imaging system comprises a small control unit and a camera unit (PDE, Hamamatsu Photonics, Hamamatsu, Japan). The camera unit is composed of a charge-coupled device camera, which filters out light with wavelengths of under 820 nm, and 36 light-emitting diodes with a wavelength of 760 nm.

ICG-Fluorescent Imaging on the Liver Surface Prior to Resection

Following the mobilization of the liver, the camera imaging head is positioned about 20 cm above the liver surface, the surgical lights are turned off (the ceiling lights were kept on), and the fluorescent images on the liver surfaces are displayed on a television monitor in the operation room (Fig. **1**).

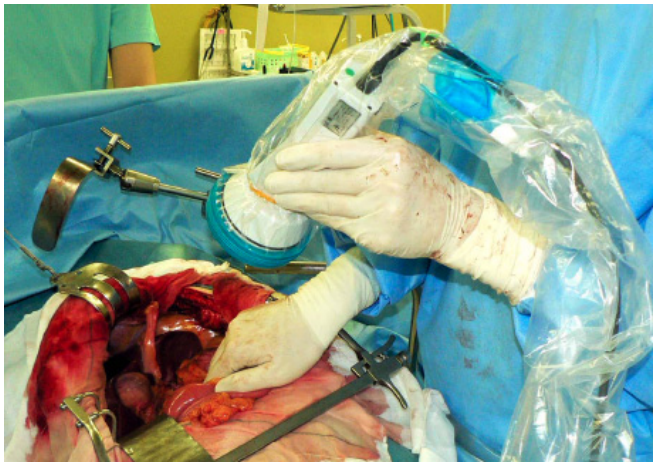

Fig. (1). Photograph of the operative field during ICG-fluorescent imaging.

ICG-Fluorescent Imaging of the Surgical Specimens

In the operation room, surgical specimens are cut to include each tumor's maximum diameter based on gross inspection immediately after the liver resection. The remaining parts of the specimens are also sliced into 10-mm sections using a long blade. The camera imaging head is positioned 20 cm above the surgical specimen, which is set in a lightproof box, and the fluorescent images of the surgical specimens are displayed on a television monitor. When the fluorescent imaging detects new lesions, the fluorescing areas are marked with needles for subsequent microscopic examinations.

ABILITIES OF ICG-FLUORESCENT IMAGING TO IDENTIFY LIVER CANCERS

ICG-Fluorescent Imaging on the Liver Surface Prior to Resection

Because of the limited tissue penetration of near-infrared light (up to 5 - 10 mm [23] the major expected role of the ICG-fluorescent imaging on liver surfaces is to detect small liver cancers located just beneath the liver surface or the raw surface before and during liver transection, compensating for the drawback of intraoperative ultrasonography in visualizing such lesions. In our previous series, this technique identified 21 of the 41 HCCs (51%) and all of the 16 CRC metastases on the liver surface prior to the resection (Fig. **2**) [20] The lower detectability in HCCs compared with that in metastases probably depended on the depth of the tumors from the liver surface, rather than the pathological characteristics: among the 20 tumors that were not identified by intraoperative ICG-fluorescent imaging, the tumor location was significantly deeper than that of the 37 identifiable tumors, and more than 90 % of tumors located within 10 mm from the liver surface were identifiable by fluorescent imaging [20]. This technique may also be useful for detecting residual cancerous tissues on the raw surface of the remnant liver after resection.

ICG-Fluorescent Imaging of the Surgical Specimens

Since near-infrared light penetrate human tissues up to 5-10 mm as described previously [23] ICG-fluorescent imaging would theoretically enable identification of all liver cancers if the surgical specimens are sliced into 10-mm sections. Indeed, in our previous series, this technique identified all of the microscopically confirmed HCCs (n = 63) and CRC metastases (n = 28) on cut surfaces of the resected specimens [20]. Furthermore, 8 of the 63 HCCs (5 of the 11 early HCCs) were not evident grossly unless visualized by ICG-fluorescent imaging because of their deep tumor locations and/or indistinct tumor margins. Gotoh *et al.* also reported that ICG-fluorescent imaging detected grossly unidentifiable HCCs in 4 out of 10 patients who underwent liver resection [24]. These results indicate that ICG-fluorescent imaging enables highly-sensitive identification of small liver cancers, especially early HCCs, during liver resection and the subsequent macroscopic examination of the resected specimens.

MECHANISMS FOR THE ICG-FLUORESCENT IMAGING OF LIVER CANCERS

The fluorescent patterns of liver cancers on their cut surfaces are classifiable into a total fluorescent type (all of the cancer tissues showed a uniform fluorescence), a partial fluorescent type (part of the cancer tissues showed fluorescence), and a rim fluorescent type (the cancer tissues were negative for fluorescence but the surrounding liver parenchyma showed fluorescence, Fig. **3**). In the previous study, we demonstrated that the fluorescent patterns are closely associated with characteristics of liver cancers: the total fluorescent-type tumors included all of the well-differentiated HCCs, while the rim fluorescent type-tumors consisted of only poorly differentiated HCCs and CRC metastases [20]. Furthermore, fluorescent microscopy confirmed the presence of fluorescence in the cytoplasm and pseudoglands of the HCC cells and in the non-cancerous liver tissues surrounding the poorly differentiated HCCs and metastases. These results suggest that well- or moderately differentiated HCCs exhibit tumor fluorescence, because the cancer tissues retain the portal uptake of ICG despite the functional and/or architectural destruction of the biliary excretion pathway because of cancer progression. In contrast, poorly differentiated HCCs and metastases produce rim fluorescence, probably because of the biliary excretion disorder in the surrounding non-cancerous liver tissue that has been compressed by the tumor. Such mechanisms underlying the ICG-fluorescent imaging of liver cancers are consistent with those for delayed (10 - 24 hours) magnetic resonance imaging using contrast material excreted *via* bile, which has been used to demonstrate tumorous hyperenhancement in well-differentiated HCCs and rim enhancement in metastases [25-28].

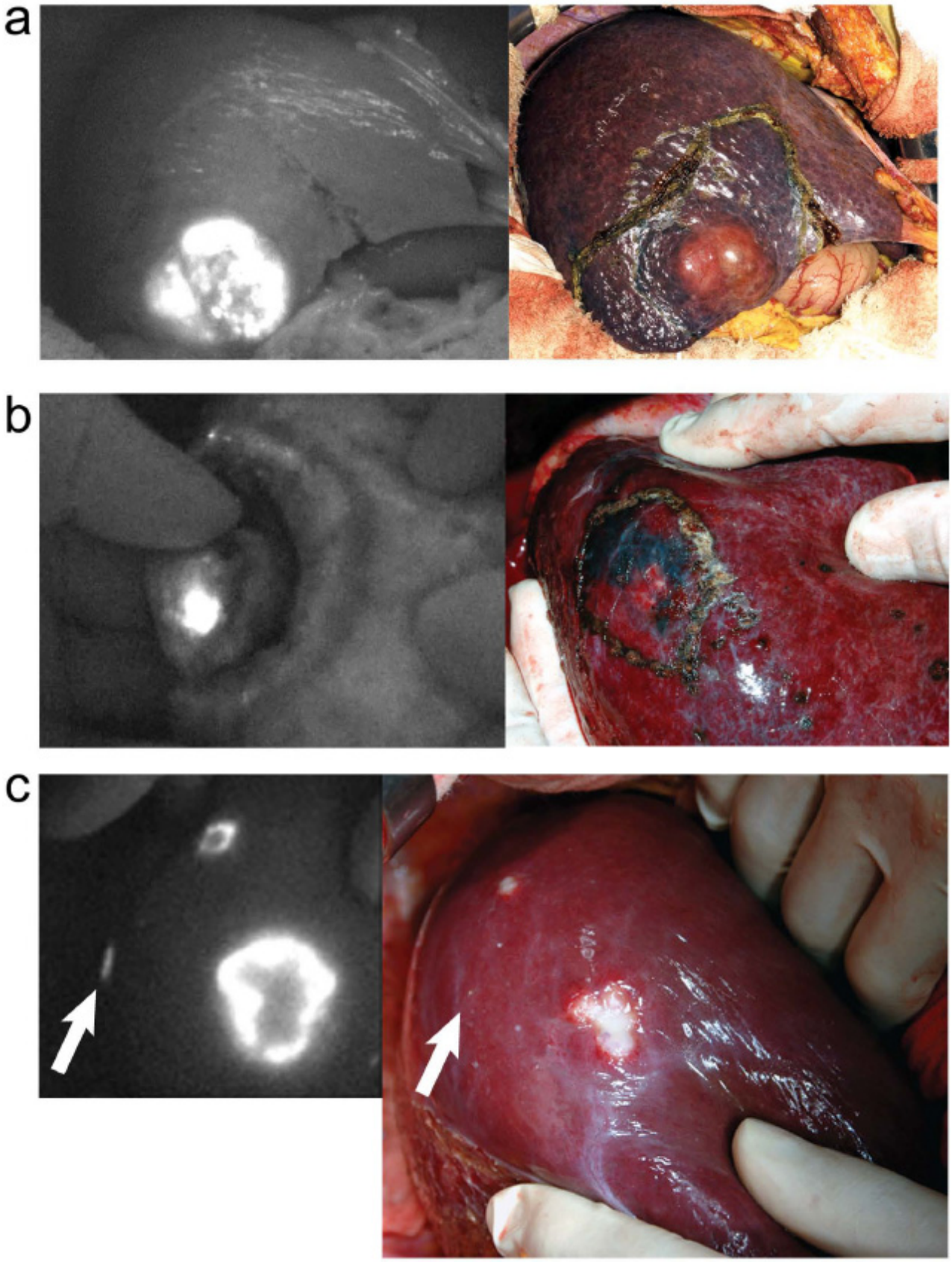

Fig. (2). ICG-fluorescent imaging on liver surfaces prior to resection (left) and their gross appearances (right). (**a**) Fluorescent imaging clearly identified HCC on the liver (segment V). (**b**) Liver resection was proceeded confirming location of HCC, which was fluorescing on the liver surface (segment VI, please see Supplementary Video 1). (**c**) Fluorescent imaging enabled visualization of the metastasis of CRC that was palpable but grossly unidentifiable (arrow) as well as the other 2 lesions exposing on the liver surfaces.

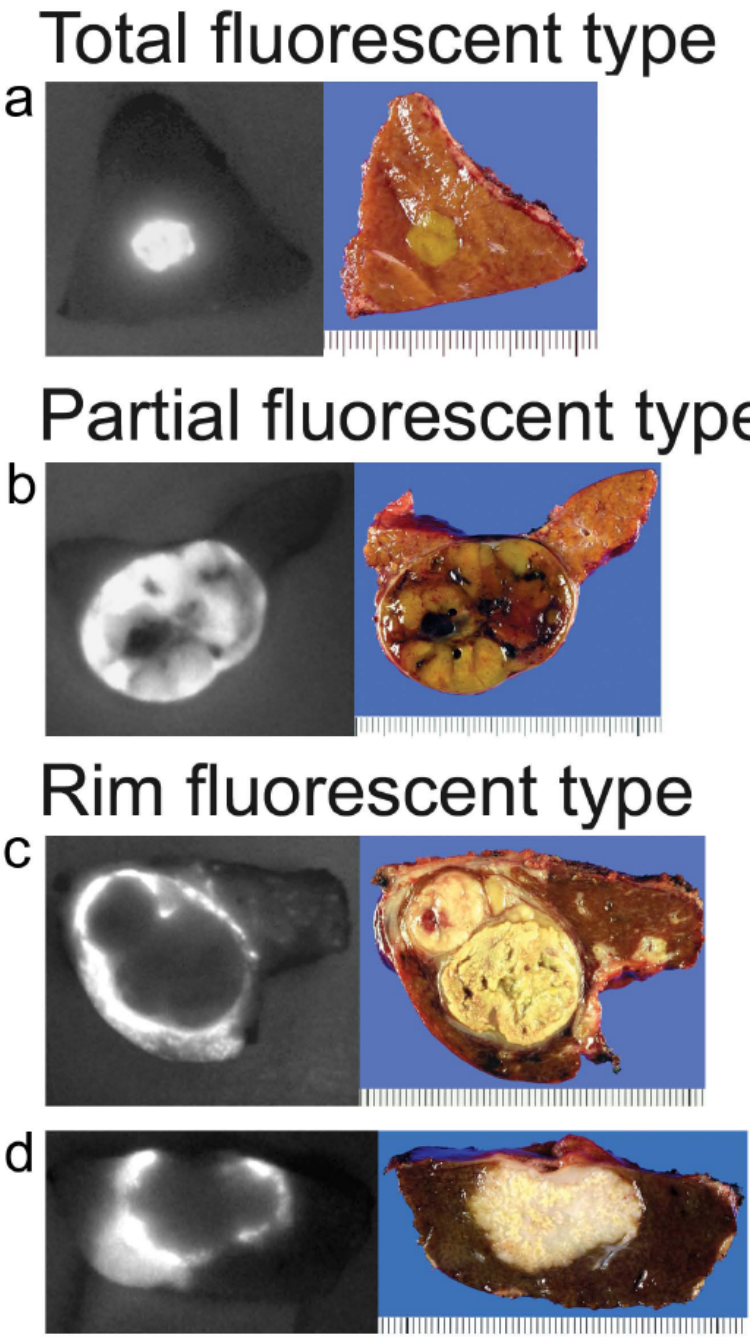

Fig. (3). Fluorescent patterns of liver cancers on cut surfaces (left) and their gross appearances (right). (**a**) Total fluorescent type (well-differentiated HCC, 7 mm in diameter). (**b**) Partial fluorescent type (moderately differentiated HCC, 35 mm in diameter). (**c**) Rim fluorescent type (poorly differentiated HCC, 30 mm in diameter). (**d**) Rim fluorescent type (metastasis of CRC, 25 mm in diameter).

ADVANTAGES OF ICG-FLUORESCENT IMAGING OF LIVER CANCERS

The major advantages of the ICG-fluorescent imaging techniques are its safety and feasibility. ICG is already used worldwide to evaluate liver function prior to resection. The reported incidence of adverse reactions following intravenous injection of ICG is quite small (approximately 0.003%) [29]. Furthermore, our fluorescent imaging technique can utilize the ICG that is injected for routine liver function testing. Once ICG is administered within 14 days prior to surgery, we can obtain fluorescent images of liver cancers in real time by simply placing the camera imaging head on the liver surface or on surgical specimens.

OTHER ICG-FLUORESCENT IMAGING TECHNIQUES IN HEPATOBILIARY SURGERY

ICG-fluorescent imaging has recently been used not only to identify liver cancers but also to delineate the tumor-bearing liver segments during anatomic resection [30] and the bile duct anatomy during liver resections or cholecystectomy (fluorescent cholangiography)[31-33]. These novel fluorescent imaging techniques may also be

useful to increase the safety and accuracy of surgeries for hepatobiliary cancers.

FUTURE PERSPECTIVES

Recently, laparoscopic hepatectomy has become prevalent worldwide [34-37]. However, identification of liver cancers during laparoscopic settings is sometimes difficult because laparoscopy offers extremely limited visualization and palpability of the liver surface as compared with open surgery. With the application of the fluorescent imaging system to laparoscopy [17-32]. ICG-fluorescent imaging may become an indispensable means of identifying liver cancers during laparoscopic hepatectomies [38].

Although ICG-fluorescent imaging of liver cancers has already been shown to be clinically useful for the detection of cancer lesions during liver resection, the specificity of this technique still remains unclear. The incidence and characteristics of false-positive lesions should be clarified in larger study populations to determine whether or not we should resect the new lesions, that have not been identified by preoperative imaging studies, detected by fluorescent imaging. The optimal dosage of ICG for fluorescent imaging of liver cancers should also be determined in future studies based on evaluation of individual patients' liver function.

CONCLUSIONS

ICG-fluorescent imaging enables highly-sensitive identification of small and grossly unidentifiable liver cancers in real time, enhancing the accuracy of liver resection and operative cancer staging.

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