

Review Article

Indocyanine green and its nanosynthetic particles for the diagnosis and treatment of hepatocellular carcinoma

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Abstract: Indocyanine green (ICG) is an amphiphilic dye, which has been used as a diagnostic agent for decades. It is becoming increasingly utilized for the diagnosis and treatment of several diseases. Primary liver cancer is a common malignancy, particularly in China. We review the published literature describing how ICG plays increasingly important roles in the diagnosis, surgical planning and treatment of hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma, indocyanine green, diagnosis and treatment, nanoparticles, photodynamic therapy, photothermic therapy

Hepatocellular carcinoma (HCC) is the fifth most common malignant tumor and the second most common cause of cancer death worldwide [1]. In 2015, there were 854,000 people living with HCC. Over 810,000 patients died from the disease [2]. In China, HCC is the fourth and fifth most common malignant tumor in males and females, respectively [3]. Although the modalities used in the treatment of HCC, including surgery, interventional therapy, biotherapy and ablation are improving, long-term survival outcomes remain poor. HCC is prone to developing recurrence and metastasis, resulting in poor prognosis, high mortality and short patient survival. Thus, it is extremely important to explore novel diagnostic and treatment options. In this review of the published literature, we describe how indocyanine green (ICG) plays increasingly important roles in the diagnosis, surgical planning and treatment of HCC.

ICG

ICG is an intravenously administered amphiphilic cyanamide dye consisting of two polar sulfonates and tetragonal ammonium groups.

Protein-bound ICG can emit near-infrared light with a wavelength of 840 nm when stimulated by external light with a wavelength ranging from 750 to 810 nm. In 1954, it was approved by the United States Food and Drug Administration for clinical use in the measurement of cardiac output, assessment of liver function and inspection of the retina and choroidal vascular systems of the eye [4-6]. After intravenous injection of 0.1-0.3 mg/kg, ICG can be examined in a healthy subject in eight minutes [7]. There are no known negative effects of intravenous administration. The incidence of allergic reaction with its use is less than 1% [8]. In the 1970s, ICG with protein binding was found to have a strong absorptive capability in the near infrared light (NIR) spectra. Due to the contrasting low NIR spectral absorption of biological tissue, ICG is suitable for biological imaging using a contrasting signal-to-background ratio [9].

With their minimally invasive properties, photodynamic therapy (PDT) and photothermal therapy (PTT) [10, 11] are emerging diagnostic and therapeutic modalities of light therapy [12]. PDT can treat tumors with low toxicity and a nonin-

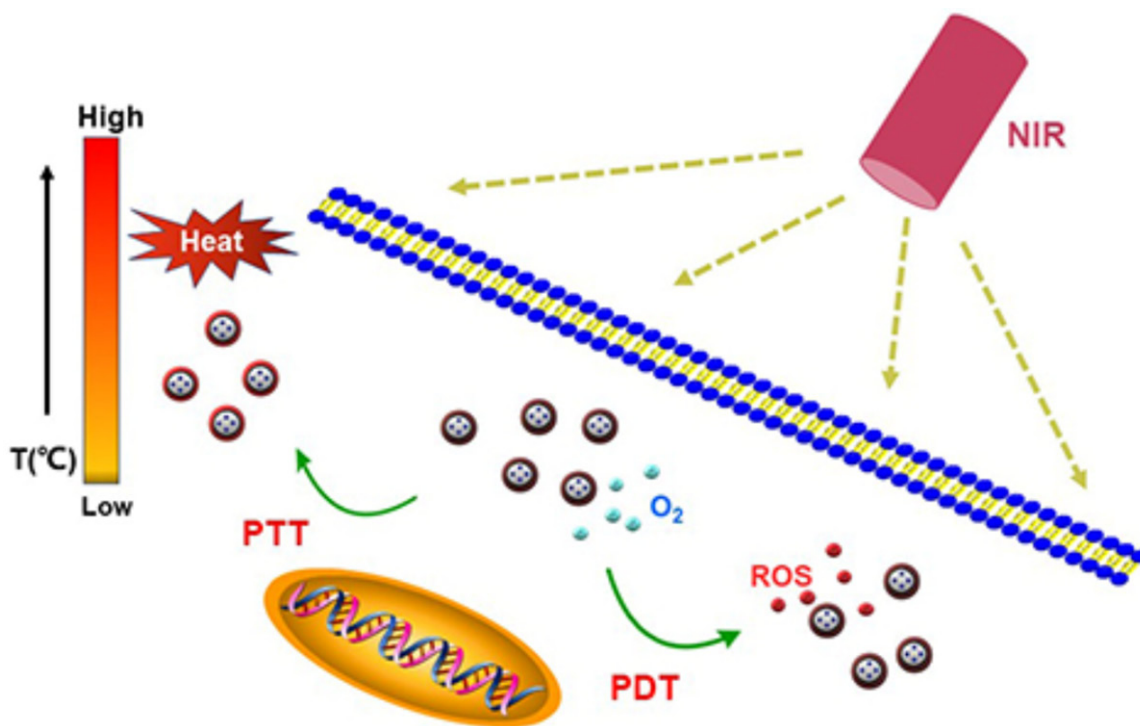


Figure 1. PDT and PTT treatment for tumor cells.

vasive nature [13]. In the treatment process, light energy is absorbed by a photosensitizer. Through an interaction between the agent and the surrounding oxygen-rich environment, electrons are elevated from the ground state to the singlet and subsequent triplet state [14, 15]. The embodiment of PDT is the resultant production of reactive oxygen species (ROS), particularly monomer oxygen species (102) [16]. ROS act as therapeutic mediums with a luminescent effect, killing cancer cells [17]. PTT is another attractive antineoplastic treatment that converts light energy into heat energy with high efficiency, high selectivity and a low side effect profile [18, 19] (Figure 1).

ICG in the diagnosis of HCC

Traditional methods of diagnosis of HCC include contrast enhanced ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) [20, 21]. Hypoechoic masses consistent with early stage HCC can be detected with a sensitivity of 63% using US [22]. The merits of US are its noninvasive and nonradiative properties; however, the drawbacks include limited visualization, large imped-

ance in some patients and a variation in quality based on the operator's skill level [23].

CT and MRI have also been widely used in HCC diagnosis. Enhanced MRI and CT are particularly useful in the detection of tumors less than 2 cm in size. Approximately 25%-30% of cases that are not visible on US can be detected using these modalities [21, 23].

ICG is a hydrophilic organic anion with a high excretion rate and stability in hepatocytes [24]. The biliary excretion of ICG utilizes phospholipid microtubules and mixed lipid/bile salt micelles. ICG is rapidly absorbed in tissue using the anion transporting polypeptide 1B3 (OATP1B3) and sodium taurocholate-transporting polypeptide (NTCP) [25]. When stimulating light is applied, fluorescence appears. After a period of time, ICG is excreted into the biliary tract through multidrug resistance-associated protein 2 (MRP2), a key gene and protein involved in ICG metabolism [26] (Figure 2). ICG does not participate in the enterohepatic circulation. Therefore, fluorescence gradually weakens. ICG is acidophobic, with a good water solubility. As the hydrosolvent of ICG has no stability, ICG

Indocyanine green in hepatocellular carcinoma

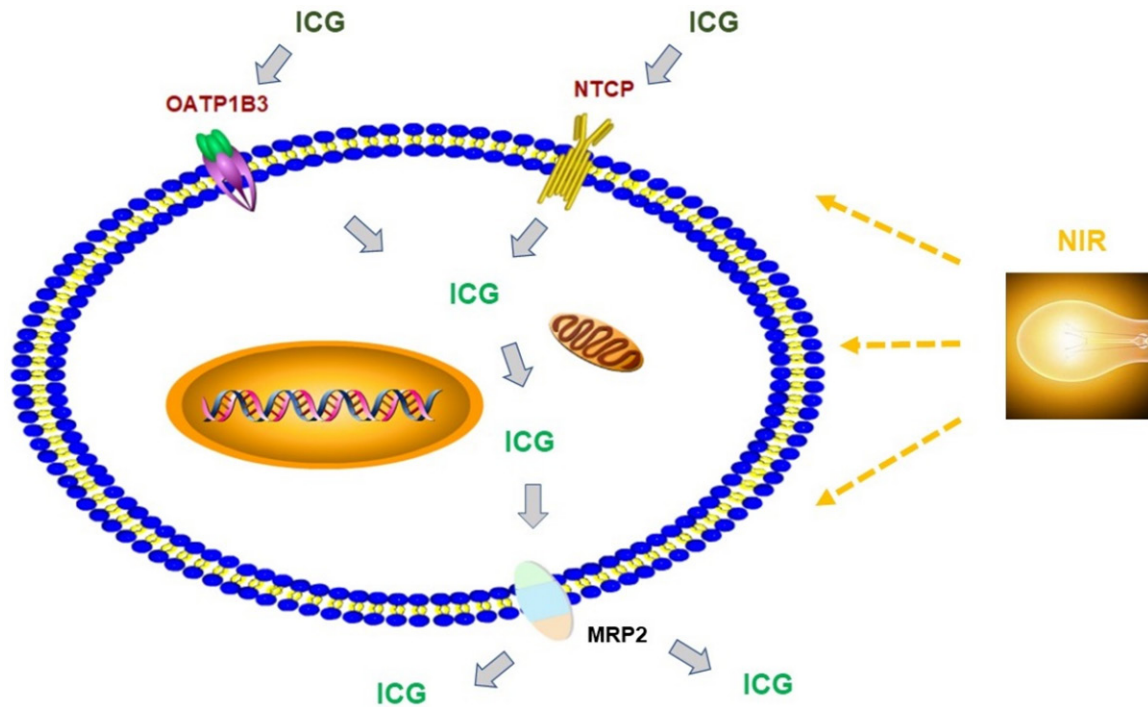


Figure 2. ICG is absorbed through OATP1B3 and NTCP in the liver cells. Once the cells are irrigated by NIR, ICG turns green; ICG is expelled from cells through MRP2.

should no longer be present in circulation six to ten hours after dissolution [27].

There is a delay of ICG discharge in parts of a liver tumor or in cirrhotic nodules with impaired liver function, resulting in a delay of fluorescence regression [28]. A well differentiated HCC can be diagnosed by its strong and homogeneous fluorescence which is due to the ability of HCC cells to absorb ICG. A poorly differentiated HCC or metastatic liver tumor is surrounded by a circular fluorescence due to impaired metabolism of ICG by tumor cells, leading to the blockage of ICG release [28, 29]. A clinical study, including 276 tumors in 170 HCC patients reported that ICG fluorescence occurred in 273 tumors. Thirty-five of these tumors were not detected prior to operation. Fourteen were found after liver resection. Twenty-one were found using ICG fluorescence in the gross resection specimens [30]. Thus, ICG can be used to diagnose small HCC at an early stage with the potential for a good cure rate [28, 31].

The limited depth of NIR irradiation means ICG can only be used to detect tumors located in

the superficial parts of the liver [32]. Also, liver tumors of less than 1 cm are difficult to detect. To overcome this, some researchers have attempted to detect small HCCs using a combination of photoacoustics and fluorescence. Au@liposome-ICG [33], ICG-loaded Au@SiO₂ [34] and ICG-HAS-Au complex (ICG-human serum albumin-Au) [35] have been used in the early diagnosis of small HCCs either pre or intra-operatively (**Table 1**).

ICG evaluation of liver function

Methods for the assessment of liver function include static and dynamic tests. Static evaluation includes: serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels for the detection of hepatic parenchymal injury [36], serum lactate dehydrogenase [37] and plasma bilirubin concentrations for the assessment of synthetic activity [38], serum gamma-glutamyl transpeptidase (GGT) level to indicate cholestasis and serum bilirubin level to evaluate excretion [39]. Dynamic evaluation includes: ICG for clearance, galactose for elimination and lidocaine for metabolite formation [37].

Table 1. Characters of ICG and ICG-Au nano-particles for diagnosis

Name	Characters
ICG [33]	Detection tumor: > 1 cm
Au@liposome-ICG [33]	Detection tumor: millimeter level
ICG-loaded Au@SiO ₂ [34]	Distance tumor to normal tissue: 0.5 mm
ICG-HSA-Au complex [35]	At least 5-fold brighter than ICG

Table 2. Characters of ICG and ICG-Au NANO-particles for treatment

Name	Characters
ICG+FA [58]/ASGPRs [60]	Tumor targeting
ICG+DOC [60]/gemcitabine [61]	Loading chemotherapy drug
DOX/ICG@Gal-HES-PCL NCs [60]	Tumor targeting+chemotherapy drug

ICG clearance was initially used to measure blood volume and cardiac output [40]. It is now widely used to assess liver function before resection and transplantation [41]. There are two indices for ICG assessment of liver function: 1) ICG plasma disappearance rate (ICG_{PDR}) and 2) ICG retention rate at 15 min (ICG_{R15}). ICG_{PDR} measures the percentage decrease in ICG blood concentration from 100% (normal > 18%/min). ICG_{R15} measures the ratio of ICG blood concentration and the incipient concentration 15 min after the ICG injection (normal range, 0% to 10%) [40].

With a less adverse reaction rate, ICG fluorescence has the clinical advantages of safety and usability [42]. Thus, it is now used to assess liver function preoperatively. As assessment of liver function using ICG_{PDR} is based on hepatic perfusion and hepatocyte metabolism, ICG_{PDR} has also been used to estimate prognosis in chronic liver diseases and failure. The method of using ICG to evaluate liver function is based on the plasma clearance rate and plasma residual rate after intravenous administration of ICG [43]. The patient's clearance is compared to the normal range of plasma clearance, which is between 18% and 24%/min [44]. ICG_{R15} correlates with Child's grading and platelet count and interrelates with in-hospital mortality and long-term survival outcomes in patients with HCC [45].

ICG for surgical planning in liver cancer

Intraoperative diagnosis and localization of small HCCs is difficult, particularly for tumors without clear boundaries. The intraoperative application of ICG has improved the detection

rate of these small lesions. It has also been used in the visualization of biliary tract anatomy [28] and mapping the lymphatic distribution [46]. The ICG fluorescence imaging system (FIS-ICG) was also used in the early 1970s for intraoperative retinography [47]. ICG is excreted into the biliary system making it a useful tool in hepatobiliary surgery [48]. A strong role in laparoscopic surgery is suggested because of the loss of tactile sensation to detect

tumors [49]. FIS-ICG has been used in cholecystectomy, benign liver tumor operations and partial liver transplantation [50].

HCC and liver metastasis from colorectal cancer are the most common primary and secondary malignant tumors of the liver. ICG fluorescence in hepatectomy can help to define the edge of the tumor from the normal liver tissues prior to or during resection [51, 52]. Studies have indicated that normal liver and tumor tissues can be distinguished by administering 7.5 mg of ICG. However, there has not been a consensus on the timing of administration [53, 54]. Some researchers have shown that the time interval between injection and operation may even be longer after administration of ICG at a dosage of 0.5 mg/kg [55]. ICG secretion becomes dysfunctional once ICG enters the liver, leading to the development of fluorescence imaging. With current technology, fluorescence imaging cannot penetrate deeply enough to show tumors of more than 8 mm from the liver surface, limiting its use in deeper seated lesions [29].

Treatment of HCC by ICG nanosynthesis

Many nanoparticles have been developed for photoacoustic and fluorescence dual-mode imaging, including ICG liposomes [56], ICG liposome/gold nanoparticle hybrids [33] and ICG-loaded Au@SiO₂ [34]. These could be used not only for pre-operative diagnosis, but also for intra-operation navigation.

The non-toxic characteristics of ICG have led researchers to also generate derivative compounds based on ICG to treat liver cancer.

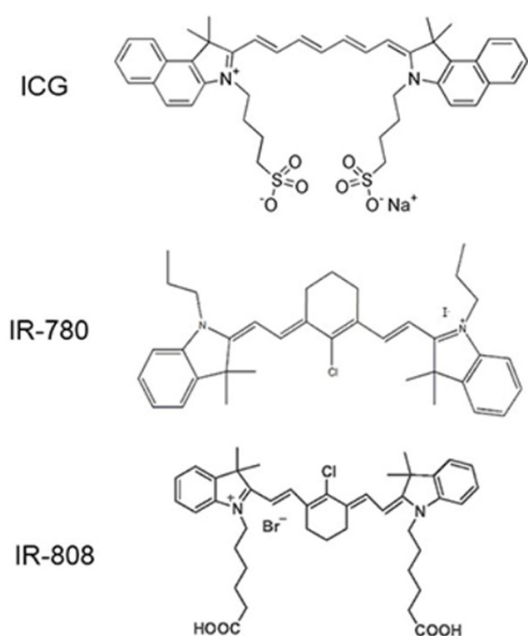


Figure 3. Chemical formula of ICG, IR-780 and IR-808.

Kaneko *et al.* demonstrated after ICG uptake by HuH-7 and HepG2 HCC cell lines, followed by NIR irradiation and photodynamic therapy, that growth of tumor cells was suppressed. Under microscopy, the nuclei of HCC cells became dense, the intercellular space increased and cell pyknosis followed [57]. In recent years, many nanoparticles have been developed for oncological treatment, including the various forms of nanoparticles for photothermal therapy (PTT), including carbon nanomaterials, organic NIR dyes and gold nanoparticles [58]. As ICG is a PTT agent with rapid degradation and clearance, nanomaterials with ICG have been increasingly used in malignant tumors, especially liver cancer.

Folic acid receptors (FR) are highly expressed on HCC cell membranes. As binding of folic acid (FA) to FR is highly effective, FA can be used to treat HCC [59]. Wang *et al.* generated FA-Janus Silver/Silica Nanoplatfoms (FA-JNPs@ICG) and found FA-JNPs@ICG could especially kill HCC cells by FR-mediated endocytosis [58]. Hu *et al.* synthesized doxorubicin and indocyanine green@galactose-functionalized hydroxyethyl starch-polycaprolactone nanoparticles (DOX/ICG@Gal-HES-PCL NCs), which were used to kill liver cancer by PTT and chemotherapy based on asialoglycoprotein receptors (ASGPRs) endocytosis [60]. Inagaki *et al.* generated a conju-

gated compound of ICG with gemcitabine. This compound has the advantages of low toxicity to normal cells and superior anti-liver tumor action in HuH-7 and HepG2 cell lines, compared with gemcitabine alone [61] (**Table 2**). Recently, Huang *et al.* used a method of phosphatase-triggered self-assembly to form a tail to end structure of ICG which can be used to load drugs improving their biological characteristics [62].

The above-mentioned ICG derivatives can potentially be used to treat liver cancer. Further development of these derivatives in liver cancer treatment requires more studies.

IR-780 and IR-808

IR-780, a photosensitizer both for PTT and PDT, is a small fluorescent molecule of heptamethylene cyanine. It has a similar structure to ICG with similar characteristics in tumor targeting and near-infrared light absorption [63]. IR-780 also shares the same process of hepatic metabolism and bile duct excretion [64].

IR-808, firstly synthesised by Chen *et al.*, is an advanced cyanine with hydrophilic improvement and stability based on IR-708 [65]. It has an maximum absorption band at 808 nm. Chen *et al.* used murine models and demonstrated that, after caudal vein injection, IR-808 was first stored in the liver and heart. After 48 hours, almost all the IR-808 was expelled from the body. In the xenograft tumor model, fluorescence could be still detected at 10 days after injecting IR-808 alone [66]. IR-808 could be used as oncotarget ligands to load anticancer drugs for tumor imaging and treatment [67] (**Figure 3**).

Conclusions

ICG is a safe, increasingly utilized diagnostic reagent that can be used to outline HCC in situ and evaluate liver function. It plays a vital role in HCC surgical mapping. Some nanoparticles that have the potential to treat HCC have been fabricated based on ICG. Future research and subsequent development of nanomaterials to specifically treat HCC are needed.

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Indocyanine green in hepatocellular carcinoma

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Disclosure of conflict of interest

None.

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Indocyanine green in hepatocellular carcinoma

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Indocyanine green in hepatocellular carcinoma

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