Detection of Peritoneal Dissemination with Near-infrared Fluorescence Laparoscopic Imaging Using a Liposomal Formulation of a Synthesized Indocyanine Green Liposomal Derivative

ISAMU HOSHINO[1], TETSURO MARUYAMA[1], HIROMICHI FUJITO[2], YUTAKA TAMURA[3], AKIKO SUGANAMI[3], HIDEKI HAYASHI[2], TARO TOYOTA[4], YASUNORI AKUTSU[1], KENTARO MURAKAMI[1], YUKA ISOZAKI[1], NAOKI AKANUMA[1], NOBUYOSHI TAKESHITA[1], TAKESHI TOYOZUMI[1], AKI KOMATSU[1] and HISAHIRO MATSUBARA[1]

Departments of [1]Frontier Surgery and [3]Bioinformatics, Graduate School of Medicine, Chiba University, Chiba, Japan; [2]Center for Frontier Medical Engineering, Chiba University, Chiba, Japan; [4]Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University, Chiba, Japan

Abstract. *Background/Aim: Although conventional staging laparoscopy (SL) has improved the diagnostic accuracy of peritoneal dissemination, novel technology is needed to increase the sensitivity of SL. We herein describe a new imaging method employing near-infrared (NIR) fluorescence imaging using a liposomal synthesized indocyanine green (ICG) liposomal derivative, LP-ICG-C18. Methods and Results: LP-ICG-C18 is a NIR-photoactivating probe in which an ICG fluorophore is covalently conjugated with a phospholipid moiety. Nude mice were intraperitoneally injected with gastric cancer cells. Twelve days later, the mice were given intravenous injections of LP-ICG-C18 at a dose of 0.15 mg/kg. A NIR imaging system was used to identify the disseminated tumors. The disseminated nodules in mice were detected without any difficulties. Disseminated tumor nodules were collected from mice with or without injections of liposomal formulation and were transferred into the swine peritoneal cavity. The nodules in the swine peritoneal cavity were clearly and promptly defined by the NIR imaging system. Conclusion: NIR-fluorescing liposomal probes can effectively target peritoneal disseminated tumors and can be easily detected by a NIR imaging system. These results warrant future clinical trials of our imaging system and may contribute to a more precise diagnosis and therapeutic approach for gastric cancer patients.*

Correspondence to: Isamu Hoshino, Department of Frontier Surgery, Graduate School of Medicine, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba 260-8670, Japan. Tel: +81 432262110, Fax: +81 432262113, e-mail: i_hosino@chiba-u.jp

Key Words: Staging laparoscopy, near-infrared fluorescence imaging, indocyanin green, liposome, dissemination.

Despite developments in diagnostic modalities, many patients are exposed to unnecessary laparotomy that increases health risk rates as the morbidity associated with non-therapeutic laparotomy is not negligible. The sensitivity to detect peritoneal metastasis was reported to be low, at 28.8%, 9% and 30% by computed tomography (CT), abdominal ultrasound (AUS) and positron emission tomography (PET), respectively. Among the various methods, staging laparoscopy (SL) has been helpful to reduce the avoidable laparotomy rates by identifying patients with unrecognized incurable disease. Although the use of SL provided many benefits for cancer patients, altering treatment in 8.5-59.6% or avoiding laparotomy in 8.5-43.8% of cases, the sensitivity for detecting peritoneal metastasis with SL was reported to be 73.7-89% (Table I) (3, 6, 13, 14, 20, 21, 39). Optimization of this technology is required to increase the sensitivity of SL.

Near-infrared (NIR) fluorescence imaging laparoscopy is a technology that contributes additional anatomical and physiological information during laparoscopic surgery (4, 7, 26, 29, 40). The use of indocyanine green (ICG) as an infrared fluorescence probe is one of the major procedures used for NIR imaging. NIR imaging with ICG provides simultaneous, real-time and high-resolution identification of bile ducts and hepatic arteries during biliary tract surgery or sentinel lymph nodes during gastric cancer surgery (4, 7, 29, 40). In the present study, we evaluated the effectiveness of a liposomally

Table I. *Staging laparoscopy for gastric cancer.*

	Study by (Ref #)	Year	N	Sensitivity (%)	Specificity (%)
1	Arnold (2)	1999	49	81.8	100
2	Yano (3)	2000	32	86.7	100
3	Blackshaw (4)	2003	100	88	83
4	Lee (5)	2005	250	77	94
5	Nakagawa (6)	2007	100	73.7	100
6	Muntean (7)	2009	45	89	100

formulated novel NIR-fluorescent probe, which contains a ICG fluorophore covalently conjugated with a phospholipid moiety, for detecting intraperitoneal dissemination during SL.

Materials and Methods

Cell culture. The human gastric cancer cell lines MKN45 and KATOIII, purchased from the Japanese Cancer Research Resources Bank (address) were cultured in DMEM (Life Technologies, Grand Island, NY) supplemented with 1% streptomycin, penicillin and 10% FCS at 37˚C in a humidified atmosphere containing 5% CO_2.

Animals and peritoneal transplantation of gastric cancer cells. Seven-week-old female athymic nude mice (BALBc nu/nu) were purchased from Japan SLC (Hamamatsu, Japan). The mice were housed in laminar flow cabinets under specific pathogen-free conditions in facilities approved by the Chiba University. The MKN45 and KATOIII cells were grown to sub-confluence and cell suspensions of 1×10^6 cells/0.2 ml were transplanted into the peritoneal cavity or subcutaneously onto the posterior portion of both thighs of the nude mice.

Reagents. The near-infrared fluorescence probe using indocyanine green (ICG) as a fluorescent dye was developed in the Center for Frontier Medical Engineering, Chiba University, as described previously. (30, 34) Briefly, 1,2-Dioleoyl-3-sn-glycero-phosphocholine (10.0 mM; NOF, City, Japan), cholesterol (1.0 mM; NOF), phosphatidylethanolamine-N-methoxy-polyethyleneglycol (5000)-dioleoyl-glycero ammonium salt (5.0×10^{-1} mM; NOF) and ICG or one of its derivatives (3.2×10^{-4} mM) were dissolved in a mixed organic solvent of chloroform/methanol (volume ratio: 9/1). A thin lipid film was formed by removal of the solvent under reduced pressure. After the addition of saline at room temperature, the liposome dispersion was filtered through a 0.2-μm pore filter made of polycarbonate (Millipore, City, abbreviated State, USA). The liposomal size distribution was determined by dynamic light scattering measurement (UPA-UT151; NIKKISO, City, Japan). To determine the ratio of ICG or ICG-C18 leaking from the liposomes (34), we used a preparative gel permeable chromatographic column (PD-10; GE Healthcare, City, abbreviated State, USA) and measured the absorbance of the obtained fraction (200 μl) separated from the liposomal dispersion at 805 nm using an absorbance spectrophotometer (UV-1800; Company, Shimadzu, Japan).

Investigation of LP-ICG-C18 uptake by normal organs and/or accumulation into the tumor. The mice were injected with 100 μl suspensions of LP-ICG-C18 or ICG. Twenty-four hours after injection, mice were killed and laparotomy was performed. Then, all organs or tumors were harvested and observed by the NIR-fluorescence laparoscopic imaging system (Olympus Corp., Tokyo, Japan).

Detection of the peritoneal dissemination in the gastric cancer mouse xenograft model. The mice that received peritoneal implants of MKN45 and KATOIII cells were divided in two groups 12 days after the implantation of cells. Twelve mice (three per group) were injected with 100 μl suspensions of LP-ICG-C18 or PBS (phosphate-buffered saline). Twenty-four hours after injection, optical imaging was performed on a Xenogen IVIS 200 small animal imaging system (Xenogen, Alameda, CA, USA) equipped with an ICG band pass filter set (810-875 nm). Then, the mice were killed and laparotomy was performed. The disseminated implants in the peritoneal cavity were detected by the NIR-fluorescence laparoscopic imaging system (Olympus Corp.).

Detection of the peritoneal tumors in a porcine model. A pig underwent standardized general anesthesia with a carbon dioxide pneumoperitoneum of 10 mm Hg. Then, 0.05 mg/kg of ICG was intravenously injected preoperatively for creating the background of fluorescent signals. A first 10 mm trocar was inserted and investigated the abdominal cavity. Then a second 10 mm trocar were placed in the right lower quadrant. The disseminated tumors collected from mice, which were observed to have fluorescence signals by the NIR fluorescence imaging system, were scattered into the porcine abdominal cavity by an assistant. These scattered tumors were then examined by the surgeon under the guidance of the NIR-fluorescence laparoscopic imaging system.

Results

Faint uptake of LP-ICG-C18 in normal organs. All organs harvested after ICG or LP-ICG-C18 administrations were explored by the NIR fluorescence imaging system. After ICG administration, a faint fluorescent signal was detected in the lung. On the contrary, a variety of organs except for the brain and stomach have weak fluorescent signals after LP-ICG-C18 injection (Figure 1A).

Besides, the intensity of fluorescence in the tumors was obviously stronger than that in any normal organs after LP-ICG-C18 injection (Figure 1B).

The NIR fluorescence liposomal formulation of ICG-C18 demonstrated tumor specificity in vivo. Whole-body dynamic optical imaging of mice disseminated with human gastric tumor xenografts was performed after the systemic administration of LP-ICG-C18. Figure 2 shows a representative *in vivo* NIR fluorescence image of MKN45 and KATOIII cells in tumor–xenografted mice 24 h after intravenous administration of 100 μl suspensions of LP-ICG-C18. The Xenogen IVIS 200 small animal imaging system detected a specific uptake of LP-ICG-C18 in the tumor (Figure 2). The intensity was stronger in tumors compared to the surrounding normal tissues. A week after injection, significant fluorescence was still detected in the tumors of mice treated with LP-ICG-C18 (data not shown).

The disseminated implants in the peritoneal cavity were also detected by the NIR fluorescence imaging system. After laparotomy in the mice, the peritoneal dissemination of gastric cancer cells was observed by a conventional white light imaging system or the NIR fluorescence imaging system. Even a distant view could define the peritoneal dissemination when the NIR fluorescence imaging system was used, while the short-range view could identify tiny disseminations with sizes <1 mm (Figure 3). Although the fluorescence signal intensity of LP-ICG-C18 in the normal joints were relatively high, the intensity of background fluorescence signals in the abdominal walls and other organs were negligible and did not interfere with the positive signals.

Detection of the peritoneal tumors in a porcine model. Both the disseminated tumors collected from mice that had been injected with LP-ICG-C18 and those from mice injected with PBS were scattered into the porcine abdominal cavity by an assistant. The peritoneal dissemination of cancer cells was observed by conventional white-light imaging and the NIR fluorescence imaging system. Tumors from mice that had been injected with LP-ICG-C18 were easily detected by the NIR fluorescence laparoscopic imaging system, while those injected with ICG for creating background signals did not affect the signals from tumors (Figure 4). On the other hand, control, non-fluorescent tumors were difficult to define by both the conventional white light and NIR fluorescence laparoscopic imaging systems.

Discussion

SL has been a significant beneficial modality that has changed the management, *i.e.* avoidance of unnecessary laparotomy, in patients with gastric cancer or other malignancies (13). Although the usefulness of laparoscopy for diagnosing peritoneal disseminations is clear, the sensitivity is still unsatisfactory (3, 6, 14, 20, 21, 39). The development of a technology, such as a high-resolution laparoscopy or the acquisition of new surgical techniques, might help resolve this issue. However, an innovative technology may be necessary to change the situation dramatically. The development of an intraoperative molecular imaging system could help detect peritoneal dissemination. Indeed, a recent pre-clinical study showed that tumors in animals could be resected by a guided-surgery system using a Cy5-labeled cell-penetrating peptide conjugated to a dendrimer, which, in fact, provided a better survival outcome (22). Angiostamp®, a fluorescent RAFT-(cRGD)4 tracer molecule, has a very high affinity for the $\alpha_v\beta_3$ integrin, which is over-expressed in ovarian cancer cells (17). The use of this technique with a NIR fluorescence imaging system might contribute to the specific intraoperative detection of tumor deposits of ovarian cancer. NIR fluorescence dye, IRDye 800CW, -labeled antibodies targeting VEGF or HER2 were also reported to be highly specific and sensitive for detecting tumor lesions *in vivo* (32). Although these pre-clinical findings have encouraged future clinical studies with fluorescence imaging systems for intraoperative guided-surgery in cancer patients, none of them has yet been evaluated in the clinical setting. The LP-ICG-C18 derivative employed herein presents an advantage of being able to more rapidly enter clinical trials because its chemical structure consists of clinically-approved components.

The ICG dye was developed for NIR photography by the Kodak Research Laboratories in 1955 and was approved for clinical use in 1956 (5, 9). In the beginning, ICG was used for imaging retinal blood vessels in ophthalmology in the early 1970's (10). Recently, NIR fluorescence imaging has provided new opportunities to improve and extend the indications of sentinel lymph node (SLN) procedures (2, 8, 36, 40). The ICG dye is currently used as the accepted standard in clinical practice. Furthermore, NIR imaging with ICG is a promising technique for intraoperative tumor identification. ICG provided NIR fluorescence-based tumor localization in a limited number of hepatobiliary cancer patients, either due to physiological uptake in well-differentiated tumors or rim uptake as a result of leakage and retention in poorly-differentiated tumors and colorectal metastases (11, 12, 18, 35). ICG has several clinical advantages, which have been thoroughly verified during its long clinical use since it: (i) confers patient safety, (ii) has a short lifetime in the blood circulation, allowing repeated applications, (iii) has a good SNR (signal to noise ratio); there is not much NIR autofluorescence in tissues, giving low noise background and (iv) it can be assessed using simple and cheap imaging devices (1). However, although NIR fluorescence imaging has a recognized potential, ICG is currently the only clinically-approved NIR dye.

One of the drawbacks of using ICG is that the dye is able to pass through the target cells easily because of its relatively small hydrodynamic diameter and, thus, imaging must be

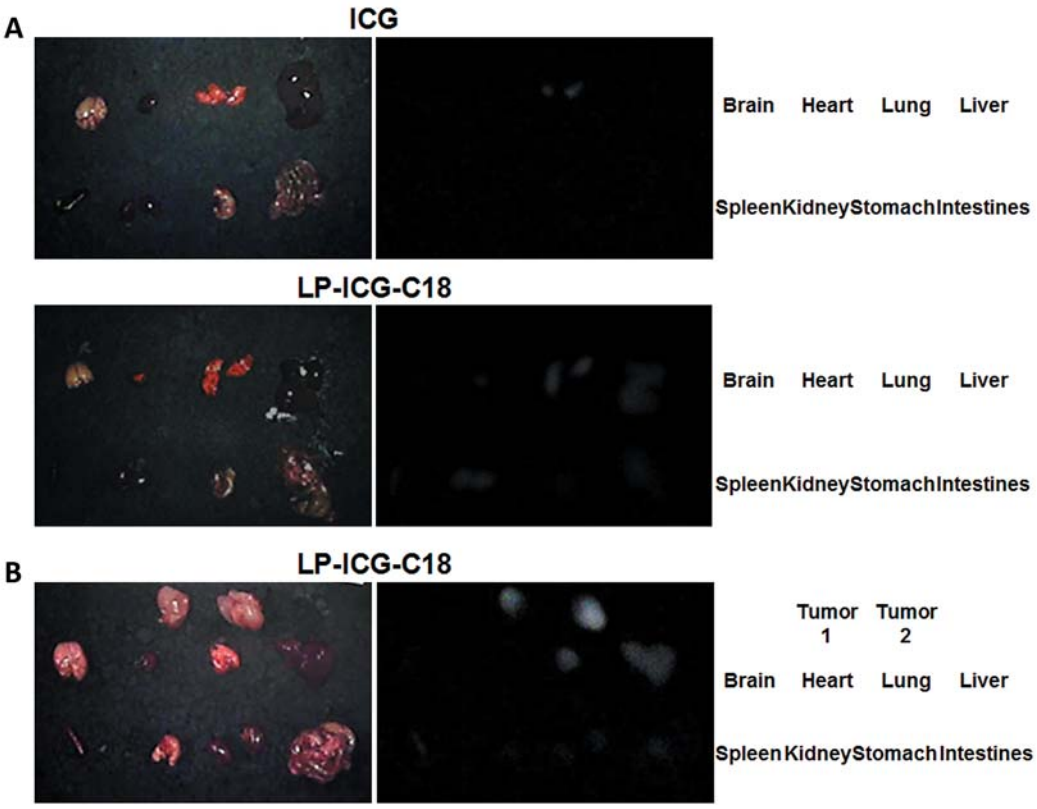

Figure 1. *Investigation of the normal organs and tumors LP-ICG-C18 uptakes. A) A faint fluorescent signal was detected in the lung. On the contrary, a variety of organs except for the brain and stomach have weak fluorescent signals after LP-ICG-C18 injection. B) The intensity of fluorescence in the tumors was obviously stronger than that in any normal organs after LP-ICG-C18 injection.*

performed shortly after ICG administration. To resolve this problem, the development of ICG derivatives has been reported. Ogawa *et al*. have conjugated ICG with several antibodies in order to target ICG to cancer cells (23). Makino *et al*. have labeled lactosome with ICG (15). The labeled lactosome was stable in the blood circulation and showed specific accumulation in liver tumors of a mouse model. Our LP-ICG-C18 also showed specific accumulation in peritoneal disseminations. Although accumulation in the normal organs was observed, it seems to be a negligible effect.

Over the past decade, several drug-loaded liposomes, lipid-based nanocarrier formulations, have been clinically approved or are under clinical investigation (25). Liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer composed mainly of natural or synthetic phospholipids. A drug can be encapsulated into these liposomes. Of interest, liposomes can be administered through parenteral, oral, pulmonary, nasal, ocular and transdermal routes (37). They are nanoparticulate carriers, usually with an 80–300 nm size range (38). Particle size is the one of the most important features of our LP-ICG-C18, which was designed to be

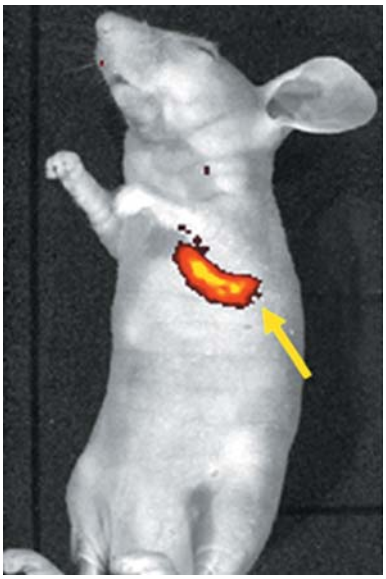

Figure 2. *Detection of peritoneal dissemination in a mouse gastric cancer xenograft model using LP-ICG-C18. Optical imaging was performed on a Xenogen IVIS 200 small animal imaging system equipped with an ICG band pass filter set (810-875 nm). The system detected the specific uptake of LP-ICG-C18 into the tumor.*

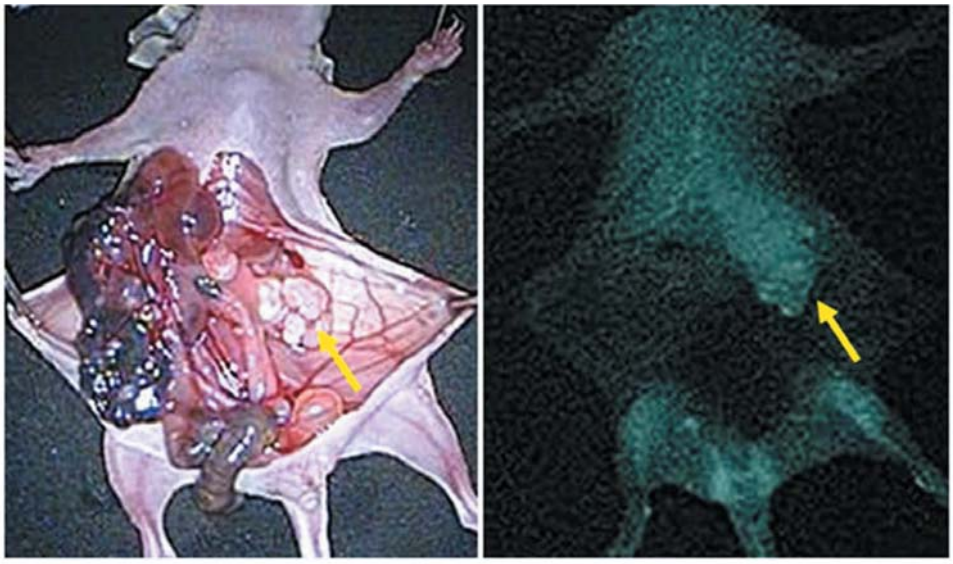

Figure 3. *Detection of the peritoneal dissemination of gastric cancer in a mouse xenograft model by conventional white light imaging or NIR fluorescent imaging. Peritoneal dissemination of gastric cancer cells was observed by a conventional white light imaging system (LEFT) or a near-infrared fluorescence imaging system (RIGHT). Even the distant view using the NIR fluorescence imaging system could define the peritoneal dissemination, while the short-range view could identify small disseminations <1 mm in diameter.*

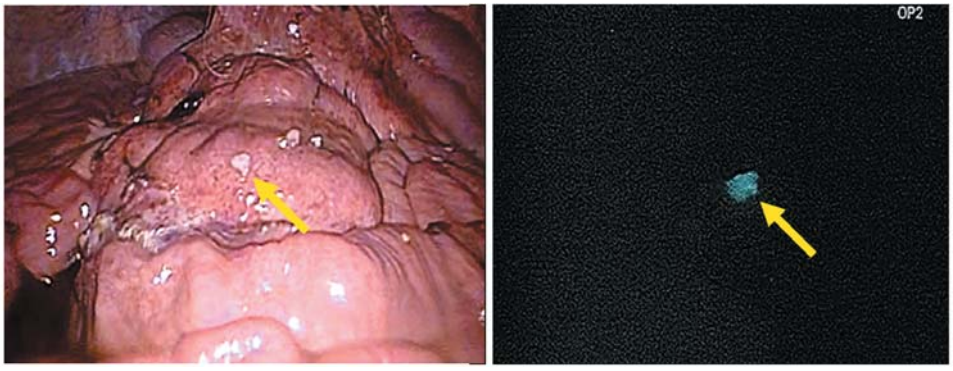

Figure 4. *Detection of peritoneal tumors in a porcine model. Tumors from mice that had been injected with the LP-ICG-C18 were easily detected by the NIR imaging system (RIGHT).*

approximately 200 nm in diameter (33). Particles, including liposomes, that are 100 nm and smaller take advantage of the enhanced permeability and retention (EPR) effect that was first described by Matsumura *et al*. (16). Currently, particles under 200 nm have been shown to have EPR effect (19, 24, 28). This effect may allow for selective accumulation of particulates in tumors (but not normal healthy tissue) because they are rich in porous blood capillaries. In addition, the tumor tissue is poor in lymphatic drainage, which enables prolonged retention of the nanoparticles. Indeed, the LP-ICG-C18 was specifically and intensely visualized in disseminated tumors even seven days after administration (data not shown).

The NIR imaging system employed herein has been clinically approved and is commercially available in Japan. There are some other commercially available systems, such as Photodynamic Eyes (PDE; Hamamatsu Photonics, Hamamatsu, Japan) (31), the SPY system (Novadaq Technologies, Concord, ON, Canada) (27), *etc*. These techniques can provide real-time visualization of anatomical structures and oncological targets during surgery. NIR imaging systems have advantages that include high tissue penetration and low autofluorescence providing, thus, sufficient contrast over the imaging other approaches. NIR fluorescent contrast agents are necessary for NIR imaging systems to visualize targets. ICG has been thought to be the

best fluorescent contrast agent and has been widely used with NIR imaging systems in surgery for various indications, including SLN mapping in multiple types of cancers, visualizing lymphatic channels, tumor imaging for "*en-bloc*" excision or angiography in reconstructive surgery (2, 8, 11, 12, 18, 35, 36, 40).

Therefore, the use of LP-ICG-C18 with a NIR imaging system can aid in the intraoperative identification of peritoneal disseminations. The extensive previous clinical experience with ICG and liposomes can lead to a rapid clinical application of LP-ICG-C18.

Conflicts of Interest

The Authors declare that there are no conflicts of interest associated with the present study.

References

1 Alander JT, Kaartinen I, Laakso A, Patila T, Spillmann T, Tuchin VV, Venermo M and Valisuo P: A review of indocyanine green fluorescent imaging in surgery. Int J Biomed Imaging *2012*: 940585, 2012.

2 Ankersmit M, van der Pas MH, van Dam DA, and Meijerink WJ: Near infrared fluorescence lymphatic laparoscopy of the colon and mesocolon. Colorectal Dis *13(Suppl 7)*: 70-73, 2011.

3 Arnold JC, Neubauer HJ, Zopf T, Schneider A, Benz C, Adamek HE and Riemann JF: Improved tumor staging by diagnostic laparoscopy. Z Gastroenterol 37: 483-488, 1999.

4 Ashitate Y, Stockdale A, Choi HS, Laurence RG and Frangioni JV: Real-time simultaneous near-infrared fluorescence imaging of bile duct and arterial anatomy. J Surg Res *176*: 7-13, 2012.

5 Bjornsson OG, Murphy R and Chadwick VS: Physiochemical studies of indocyanine green (ICG): absorbance/concentration relationship, pH tolerance and assay precision in various solvents. Experientia *38*: 1441-1442, 1982.

6 Blackshaw GR, Barry JD, Edwards P, Allison MC, Thomas GV and Lewis WG: Laparoscopy significantly improves the perceived preoperative stage of gastric cancer. Gastric Cancer *6*: 225-229, 2003.

7 Cahill RA, Ris F and Mortensen NJ: Near-infrared laparoscopy for real-time intra-operative arterial and lymphatic perfusion imaging. Colorectal Dis *13(Suppl 7)*: 12-17, 2011.

8 Can MF, Yagci G and Cetiner S: Sentinel lymph node biopsy for gastric cancer: Where do we stand? World J Gastrointest Surg *3*: 131-137, 2011.

9 Engel E, Schraml R, Maisch T, Kobuch K, Konig B, Szeimies RM, Hillenkamp J, Baumler W and Vasold R: Light-induced decomposition of indocyanine green. Invest Ophthalmol Vis Sci *49*: 1777-1783, 2008.

10 Flower RW: Injection technique for indocyanine green and sodium fluorescein dye angiography of the eye. Invest Ophthalmol *12*: 881-895, 1973.

11 Gotoh K, Yamada T, Ishikawa O, Takahashi H, Eguchi H, Yano M, Ohigashi H, Tomita Y, Miyamoto Y and Imaoka S: A novel image-guided surgery of hepatocellular carcinoma by indocyanine green fluorescence imaging navigation. J Surg Oncol 100: 75-79, 2009.

12 Ishizawa T, Fukushima N, Shibahara J, Masuda K, Tamura S, Aoki T, Hasegawa K, Beck Y, Fukayama M and Kokudo N: Real-time identification of liver cancers by using indocyanine green fluorescent imaging. Cancer 115: 2491-2504, 2009.

13 Leake PA, Cardoso R, Seevaratnam R, Lourenco L, Helyer L, Mahar A, Law C and Coburn NG: A systematic review of the accuracy and indications for diagnostic laparoscopy prior to curative-intent resection of gastric cancer. Gastric Cancer *15(Suppl 1)*: S38-47, 2012.

14 Lee YT, Ng EK, Hung LC, Chung SC, Ching JY, Chan WY, Chu WC and Sung JJ: Accuracy of endoscopic ultrasonography in diagnosing ascites and predicting peritoneal metastases in gastric cancer patients. Gut *54*: 1541-1545, 2005.

15 Makino A, Kizaka-Kondoh S, Yamahara R, Hara I, Kanzaki T, Ozeki E, Hiraoka M and Kimura S: Near-infrared fluorescence tumor imaging using nanocarrier composed of poly(L-lactic acid)-block-poly(sarcosine) amphiphilic polydepsipeptide. Biomaterials *30*: 5156-5160, 2009.

16 Matsumura Y and Maeda H: A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res *46*: 6387-6392, 1986.

17 Mery E, Jouve E, Guillermet S, Bourgognon M, Castells M, Golzio M, Rizo P, Delord JP, Querleu D and Couderc B: Intraoperative fluorescence imaging of peritoneal dissemination of ovarian carcinomas. A preclinical study. Gynecol Oncol *122*: 155-162, 2011.

18 Miyoshi N, Ohue M, Noura S, Yano M, Sasaki Y, Kishi K, Yamada T, Miyashiro I, Ohigashi H, Iishi H, Ishikawa O and Imaoka S: Surgical usefulness of indocyanine green as an alternative to India ink for endoscopic marking. Surg Endosc *23*: 347-351, 2009.

19 Monsky WL, Fukumura D, Gohongi T, Ancukiewcz M, Weich HA, Torchilin VP, Yuan F and Jain RK: Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factor. Cancer Res *59*: 4129-4135, 1999.

20 Muntean V, Mihailov A, Iancu C, Toganel R, Fabian O, Domsa I, and Muntean MV: Staging laparoscopy in gastric cancer. Accuracy and impact on therapy. J Gastrointestin Liver Dis 18: 189-195, 2009.

21 Nakagawa S, Nashimoto A, and Yabusaki H: Role of staging laparoscopy with peritoneal lavage cytology in the treatment of locally advanced gastric cancer. Gastric Cancer 10: 29-34, 2007.

22 Nguyen QT, Olson ES, Aguilera TA, Jiang T, Scadeng M, Ellies LG, and Tsien RY: Surgery with molecular fluorescence imaging using activatable cell-penetrating peptides decreases residual cancer and improves survival. Proc Natl Acad Sci U S A 107: 4317-4322, 2010.

23 Ogawa M, Kosaka N, Choyke PL and Kobayashi H: *In vivo* molecular imaging of cancer with a quenching near-infrared fluorescent probe using conjugates of monoclonal antibodies and indocyanine green. Cancer Res 69: 1268-1272, 2009.

24 Park JH, von Maltzahn G, Zhang L, Derfus AM, Simberg D, Harris TJ, Ruoslahti E, Bhatia SN and Sailor MJ: Systematic surface engineering of magnetic nanoworms for *in vivo* tumor targeting. Small 5: 694-700, 2009.

25 Puri A, Loomis K, Smith B, Lee JH, Yavlovich A, Heldman E and Blumenthal R: Lipid-based nanoparticles as pharmaceutical drug carriers: from concepts to clinic. Crit Rev Ther Drug Carrier Syst 26: 523-580, 2009.

26 Roberts WW, Dinkel TA, Schulam PG, Bonnell L and Kavoussi LR: Laparoscopic infrared imaging. Surg Endosc 11: 1221-1223, 1997.
27 Schaafsma BE, Mieog JS, Hutteman M, van der Vorst JR, Kuppen PJ, Lowik CW, Frangioni JV, van de Velde CJ and Vahrmeijer AL: The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. J Surg Oncol 104: 323-332, 2011.
28 Shenoi MM, Shah NB, Griffin RJ, Vercellotti GM, and Bischof JC: Nanoparticle preconditioning for enhanced thermal therapies in cancer. Nanomedicine (Lond) 6: 545-563, 2011.
29 Shussman N, Abu Gazala M, Schlager A, Elazary R, Khalaileh A, Zamir G, Kushnir D, Rivkind AI, and Mintz Y: Laparoscopic infrared imaging--the future vascular map. J Laparoendosc Adv Surg Tech A 21: 797-801, 2011.
30 Suganami A, Toyota T, Okazaki S, Saito K, Miyamoto K, Akutsu Y, Kawahira H, Aoki A, Muraki Y, Madono T, Hayashi H, Matsubara H, Omatsu T, Shirasawa H, and Tamura Y: Preparation and characterization of phospholipid-conjugated indocyanine green as a near-infrared probe. Bioorg Med Chem Lett *22*: 7481-7485, 2012.
31 Tagaya N, Yamazaki R, Nakagawa A, Abe A, Hamada K, Kubota K and Oyama T: Intraoperative identification of sentinel lymph nodes by near-infrared fluorescence imaging in patients with breast cancer. Am J Surg *195*: 850-853, 2008.
32 Terwisscha van Scheltinga AG, van Dam GM, Nagengast WB, Ntziachristos V, Hollema H, Herek JL, Schroder CP, Kosterink JG, Lub-de Hoog MN and de Vries EG: Intraoperative near-infrared fluorescence tumor imaging with vascular endothelial growth factor and human epidermal growth factor receptor 2 targeting antibodies. J Nucl Med *52*: 1778-1785, 2011.
33 Toyota T FH, Suganami A, Aoki A, Onoue K, Muraki Y, Madono T, Fujinami M, Tamura Y and Hayashi H: Near-infrared-fluorescence imaging of lymph nodes by using liposomally formulated indocyanine green derivatives. Bioorg Med Chem.
34 Toyota T, Fujito H, Suganami A, Ouchi T, Ooishi A, Aoki A, Onoue K, Muraki Y, Madono T, Fujinami M, Tamura Y and Hayashi H: Near-infrared-fluorescence imaging of lymph nodes by using liposomally formulated indocyanine green derivatives. Bioorganic & medicinal chemistry *22*: 721-727, 2014.
35 Uchiyama K, Ueno M, Ozawa S, Kiriyama S, Shigekawa Y and Yamaue H: Combined use of contrast-enhanced intraoperative ultrasonography and a fluorescence navigation system for identifying hepatic metastases. World J Surg *34*: 2953-2959, 2010.
36 van der Vorst JR, Schaafsma BE, Verbeek FP, Hutteman M, Mieog JS, Lowik CW, Liefers GJ, Frangioni JV, van de Velde CJ and Vahrmeijer AL: Randomized Comparison of Near-infrared Fluorescence Imaging Using Indocyanine Green and 99(m) Technetium With or Without Patent Blue for the Sentinel Lymph Node Procedure in Breast Cancer Patients. Ann Surg Oncol *19*: 4104-4111, 2012.
37 Wang J, Chen J, Ye N, Luo Z, Lai W, Cai X, and Lin Y: Absorption, pharmacokinetics and disposition properties of solid lipid nanoparticles (SLNs). Curr Drug Metab *13*: 447-456, 2012.
38 Wilczewska AZ, Niemirowicz K, Markiewicz KH, and Car H: Nanoparticles as drug delivery systems. Pharmacol Rep *64*: 1020-1037, 2012.
39 Yano M, Tsujinaka T, Shiozaki H, Inoue M, Sekimoto M, Doki Y, Takiguchi S, Imamura H, Taniguchi M, and Monden M: Appraisal of treatment strategy by staging laparoscopy for locally advanced gastric cancer. World J Surg *24*: 1130-1135; discussion 1135-1136, 2000.
40 Yoshida M, Kubota K, Kuroda J, Ohta K, Nakamura T, Saito J, Kobayashi M, Sato T, Beck Y, Kitagawa Y, and Kitajima M: Indocyanine green injection for detecting sentinel nodes using color fluorescence camera in the laparoscopy-assisted gastrectomy. J Gastroenterol Hepatol *27(Suppl 3)*: 29-33, 2012.

Received November 5, 2014
Revised November 16, 2014
Accepted November 25, 2014