EGFR-Targeted Fluorescent Imaging Using The Da Vinci[®] Firefly[™] Camera For Gallbladder Cancer

Jung Ha Choi

Yonsei University College of Medicine

Chang Moo Kang

Yonsei University College of Medicine

Jeong Youp Park (✉ sensass@yuhs.ac)

Yonsei University College of Medicine

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Abstract

Fluorescent imaging may aid with precise diagnosis and treatment of patients with gallbladder cancer. In this study we sought to demonstrate whether the da Vinci® surgical system and Firefly™ camera could detect EGFR-targeted fluorescent images in orthotopic mouse models of gallbladder cancer. An orthotopic mouse model of gallbladder cancer was created by injecting NOZ gallbladder cancer cells mixed with Matrigel into the gallbladder. *In vivo* imaging of subcutaneous and orthotopic gallbladder tumors was performed after injection of DyLight 650- or 800-conjugated EGFR antibody. Western blotting, flow cytometry, and confocal microscopy showed the presence of EGFR in NOZ cells, but not in HEK cells. Subcutaneous NOZ cell tumors fluoresced after injection with fluorescent EGFR antibody, but subcutaneous HEK tumors did not. Fluorescent EGFR antibody made orthotopic NOZ tumors fluoresce, with an intensity stronger than in surrounding normal tissues. Histochemical examination confirmed the location of the tumors inside the gallbladder and adjacent liver parenchyma. Fluorescent signal was also detected in orthotopic gallbladder tumors with Firefly™ camera. Our study showed that fluorescent EGFR antibodies and the Firefly camera in the da Vinci system can detect fluorescing gallbladder tumors, which demonstrates its potential use for molecular imaging based preivision surgery in near future.

Introduction

The incidence of gallbladder cancer globally is 2.2 individuals per 100,000 and 2.9 individuals per 100,000 in Korea¹⁻³. In advanced stage of the disease, the mortality rate is high. The 5-year survival rate is only 2.5%. However, when the tumor is confined to the gallbladder the survival rate is 73.5%³. Thus, early detection and treatment are important for survival. However, distinguishing malignant disease from benign inflammatory disease is difficult and often makes a diagnosis tricky. The diagnosis of gallbladder cancer after cholecystectomy often occurs incidentally because the presence of cancer is often not suspected⁴.

Fluorescent imaging may aid the precise diagnosis and treatment of patients with gallbladder cancer^{5,6}. Preclinical and clinical studies showed that when combined with endoscopy, laparoscopy and robotic surgery system fluorescent imaging aids the detection of cancer in various organs and allows its complete resection^{5,7}. Laparoscopic and robotic surgeries are already widely used for gallbladder surgery, so a fluorescent imaging technology can be easily incorporated⁸⁻¹⁰. The Firefly™ camera which can be integrated into the da Vinci® system, is a fluorescent imaging system that is currently used in clinical settings and can detect both visible and near-infrared light images. Its current applications include fluorescent cholangiography, bowel perfusion assessment, the lymph node mapping, and sentinel lymph node biopsy¹¹. To further expand use of fluorescent imaging technology to tumor detection and focus surgical resection, molecular imaging methods with several contrast agents targeting various cancer biomarkers are currently under development¹².

The epidermal growth factor receptor (EGFR) is one of the most frequently examined cancer biomarkers in fluorescent imaging because it is frequently altered in various human cancers.¹³ In normal tissues, EGFR is a regulator of epithelial tissue development and homeostasis, but in cancerous tissues, EGFR is associated with cell proliferation, migration and metastases, evasion from apoptosis, and angiogenesis¹⁴. In gallbladder cancer, EGFR is expressed in about 90% of cases, making it an attractive target for use in fluorescent imaging^{15,16}.

In this study we sought to demonstrate that the da Vinci Firefly camera and EGFR targeted antibody conjugated with fluorescent material could be used for detection of gallbladder cancer in tumor mouse models.

Results

Expression of EGFR in gallbladder cancer cells

Western blotting, flow cytometry, and confocal microscopy were performed to confirm that EGFR was expressed in gallbladder cancer cells. Fluorescent anti-EGFR was able to bind to the membranes of cancer cells. Western blotting was used to detect the expression of EGFR in NOZ cells, but not in HEK cells (Fig. 1a). Likewise, flow cytometry was used to show that anti-EGFR bound to NOZ cells, but not to HEK cells (Fig. 1b). Confocal microscopy was also used to show the presence of fluorescence foci along the membranes of NOZ cells, but not HEK cells, indicating that the fluorescent anti-EGFR bound to the cell membranes of EGFR-positive NOZ cells (Fig. 1c).

Imaging Subcutaneous Tumors With Fluorescent Anti-egfr

Subcutaneous tumors were grown for about 4 weeks after injecting the cells in the flanks of mice. Dylight 650-conjugated anti-EGFR was injected into the tail veins of the mice, and then the tumors were imaged. Twenty-four hours after injection, a strong fluorescent signal was detected in EGFR-positive NOZ cell tumors, but the signal was faint in EGFR-negative HEK cell tumors. Over time, the intensity of the fluorescent signal decreased, persisting up to 72 hours in the NOZ cell tumors, and while almost disappearance in the HEK cell tumors (Fig. 2a).

Analysis of the immunofluorescence from the anti-EGFR in resected subcutaneous tumors from the mice confirmed the expression of EGFR in NOZ cell tumors, but not in HEK cells tumors (Fig. 2b).

Imaging Orthotopic Tumors With Fluorescent Egfr Antibodies

NOZ cells mixed with Matrigel were injected into gallbladders, and after 5 weeks, Dylight 650 conjugated anti EGFR was injected into the tail veins of the mice. Twenty-four hours after injecting the antibody, each

mouse was sacrificed and the abdomen was opened. Upon gross examination, tumors were noted in the gallbladders of the mice. Fluorescent imaging detected strong intense signals from the tumors, which was lacking in surrounding normal tissues. *Ex vivo* imaging confirmed the fluorescence came from the tumors (Fig. 3). No fluorescent signal was noted in the absence of tumors. Subsequent histologic examination confirmed tumor in the gallbladder and adjacent liver parenchyma (Fig. 3). When no tumor was made histologic examination only detected inflammatory cells in the gallbladder and liver (data not shown).

Detection of fluorescence from orthotopic tumors using Firefly camera and the da Vinci system

In Vivo Imaging System in our facility could detect 650 nm fluorescence, but the Firefly camera could detect only 800 nm fluorescence. Therefore, imaging orthotopic tumors using the Firefly camera was done with Dylight 800 conjugated anti-EGFR, as mentioned above. Strong fluorescent signals from the tumor detected with the Firefly™ camera could be easily seen on screen of robotic surgery system (Fig. 4). *Ex vivo* imaging also confirmed that the fluorescence came from the tumor (Fig. 4).

Discussion

Our results showed that anti-EGFR specifically bound to gallbladder tumors and produced fluorescence, which could be detected by imaging during surgery. In vitro and in vivo fluorescent EGFR antibody could make EGFR positive gallbladder tumor fluorescent, but when no tumor was made or EGFR was not expressed, no fluorescence was emitted. In addition, using 800 nm fluorescent EGFR antibody Firefly™ camera in da Vinci® robotic surgery system could detect fluorescent signal from gallbladder tumor. Even though in this study fluorescent technology with robotic surgery system was tested, its use could be expanded to laparoscopic and endoscopic system also.

The present results implicated that fluorescent imaging technology could contribute to differentiate malignant gallbladder tumor from benign inflammatory disease, which could allow to avoid incomplete resection of incidental gallbladder cancer or misdiagnose gallbladder tumor. In this study when no tumor was made in orthotopic tumor models, only signs of inflammation was noted. Furthermore, it also might help to achieve complete resection by assessing tumor margin and lymph node metastasis⁶. Pathological R0 resection are usually determined by final pathological examination after radical cholecystectomy. However, clinical R0 is assumed by margin-negative simple cholecystectomy with no evidence of regional lymph node metastasis or grossly residual cancer tissues in preoperative image and intraoperative findings^{17,18}. In addition, considering simple cholecystectomy is regarded as feasible and safe in early gallbladder cancer^{19–21}, more appropriate selection criteria of simple cholecystectomy could be established based on the present EGFR-targeted fluorescent image.

EGFR targeted fluorescent imaging has been tested in several clinical trials including head and neck cancer²², pancreatic cancer²³, glioblastoma and glioma^{13,24}, oral cancer²⁵, and lung cancer¹³. Fluorescence-guided surgical imaging using panitumumab, an EGFR antibody, conjugated to IRDye800

was used to detect gliomas in real time²⁶, demonstrating good tumor-to-background contrast ratio. The same antibody has been used in a murine model of colorectal cancer for fluorescence-guided surgery with a strong fluorescent contrast²⁷. Additionally, the gallbladder is a good candidate for fluorescent imaging using EGFR-targeted antibodies because EGFR is overexpressed in gallbladder cancer regardless of stages²⁸. Other than EGFR, folate receptor²⁹, vascular endothelial growth factor³⁰, carcinoembryonic antigen³¹, cathepsins³², and matrix metalloproteinase³³ have been tested as targets for fluorescent imaging. More research is needed to find suitable biomarkers for fluorescent imaging of gallbladder cancer.

Various orthotopic tumor models have been used to develop new laparoscopic and robotic surgical techniques because they mimic the anatomical characteristics of tumors³⁴. Taking the current status of minimally invasive radical cholecystectomy in gallbladder cancer into account, present study further emphasizes usefulness of orthotopic tumor models in testing new surgical techniques for gallbladder cancer. In near future, more precise decision making on surgical extent according to florescent molecular images would be tested using da Vinci surgical system in orthotopic tumor models of gallbladder cancer.

In conclusion, our study showed that a fluorescent EGFR-targeted antibody can be used successfully for the diagnosis of gallbladder tumors, which in turn helps detect malignant disease. The use of Firefly™ camera in the da Vinci® robotic system allowed detection of gallbladder tumors via fluorescence, which demonstrates the potential of molecular imaging using this robotic surgical system. Further researches are needed for clinical application of molecular imaging using fluorescence technology.

Methods

Ethics statement. All animal experiments were approved by the Yonsei University Health System Institutional Animal Care and Use Committee (IACUC NO. 2020-0015) and performed in accordance with the ARRIVE guidelines.

Gallbladder cancer cell lines. The human gallbladder cancer cell line, NOZ (EGFR positive) and the human embryonic kidney cell lines, HEK (EGFR negative) were maintained in RPMI 1640 and Minimum Essential Medium containing Earle's balanced salts (MEM/EBSS) medium supplemented with 10% fetal bovine serum (Gibco, Carlsbad, USA), and an antibiotic-antimycotic solution (Gibco, Carlsbad, USA). All cells were cultured at 37°C in a 5% CO₂ incubator.

Mice. BALB/c nude mice (Central Lab Animal, Inc, Seoul, Korea), 4–5 weeks old, were used in the study. Mice were kept in a barrier facility under HEPA filtration and fed with an autoclaved laboratory rodent diet. All mouse surgical and imaging procedures were performed after anesthetization by an intramuscular injection of 60% tiletamine and zolazepam, and 40% xylazine HCl (0.02 ml) for anesthesia. Animals received antibiotics immediately prior to surgery and once per day for 3 days. The condition of the animals was monitored every day. CO₂ inhalation was used for euthanasia.

Antibody-dye conjugation. Mouse monoclonal antibodies to EGFR (199.12; Thermo Scientific, Rockford, IL, USA) were conjugated to DyLight 650 or DyLight 800 dyes (Thermo Scientific, Rockford, IL, USA) after removing bovine serum albumin (BSA) using Pierce™ Antibody Clean-up Kit (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's specifications.

Western blotting. Cells were lysed in lysis buffer containing 70 mM β -glycerophosphate, 0.6 mM sodium orthovanadate, 2 mM $MgCl_2$, 1 mM ethylene glycol tetraacetic acid, 1 mM DTT (Invitrogen, Grand Island, NY, USA), 0.5% Triton-X100, 0.2 mM phenylmethylsulfonyl fluoride, and 1% protease inhibitor cocktail (SigmaAldrich, St. Louis, MO, USA). Lysates were separated via sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked in 5% (w/v) non-fat dry milk and probed with anti-EGFR (D-8, Santa Cruz, Dallas, TX, USA) at dilution of 1:100. The immunoreactive proteins were visualized using the SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific).

Flow cytometry and confocal microscopy. A total of 2×10^6 cells were washed with phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde for 15 minutes at room temperature in the dark. The cells were washed and incubated with 1% BSA, for 1 hour at room temperature. After incubation, cells were washed with PBS and incubated with 0.5 nM anti-EGFR (199.125; Thermo Scientific, Rockford, IL, USA) for 3 hours at room temperature. Cells were then incubated with Alexa fluor 488-conjugated polyclonal goat anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, Inc. USA) for 1 hour at room temperature. After washing with PBS, fluorescence was detected with BD FACS LSRII SORP system (Becton Dickinson Company, New Jersey, USA) and confocal microscopy was performed using an LSM 700 instrument (Carl Zeiss, Oberkochen, Germany).

Subcutaneous and orthotopic mouse models of gallbladder cancer. To make the subcutaneous mouse models of gallbladder cancers, NOZ and HEK cells (2×10^6 cells) were injected subcutaneously into the flanks of BALB/c nude mice. When the size of the subcutaneous tumors grew to 10 ~ 20 mm in diameter, imaging was performed. To create the orthotopic mouse models of gallbladder cancer, 2×10^6 NOZ cells were mixed with Matrigel (Corning, Arizona, USA). The gallbladders were exposed, and injected with cell suspension using a 29-G insulin syringe (BD, USA). The abdominal walls were closed using Vicryl 6/0. Imaging was performed 5 weeks after the injection³⁵.

Fluorescent immunostaining. Formalin-fixed, paraffin-embedded tissue samples were sectioned into serial 4-mm slices and placed on microscope slides. After deparaffinization in xylene and rehydration in alcohol, tissue sections underwent antigen retrieval for 30 minutes in Tris-EDTA buffer titrated to pH 9.0 at 97°C and cooled with tap water. Nonspecific antigen reactions were blocked by a 1-hour incubation with M.O.M. mouse IgG blocking buffer. The slides were then incubated with the anti-EGFR (MA5-13319; Thermo Scientific, Rockford, IL, USA) at a dilution of 1:500 for 12 ~ 16 h at 4 °C. Slides were then incubated with Alexa fluor 488-conjugated polyclonal goat anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, Inc. USA) at a dilution of 1:100 for 1 hour at room temperature. After

washing with PBS, the slides were examined under confocal microscopy using LSM 700 instrument (Carl Zeiss, Oberkochen, Germany).

Imaging. Mice harboring subcutaneous and orthotopic tumors were injected with anti-EGFR (199.12; Thermo Scientific, Rockford, IL, USA) conjugated to DyLight 650 via the tail vein. Imaging was then performed using an IVIS®Spectrum In Vivo Imaging System (Perkin Elmer, Waltham MA, USA). For subcutaneous tumor, imaging was done on days 1, 2 and 3 after injection under anesthesia. For orthotopic tumor in each mouse, imaging was done after euthanasia, opening of the abdominal wall, and exposure of the liver and gallbladder. After the in vivo imaging of orthotopic tumors, the tumors and livers were removed from the mice, and ex vivo imaging was performed. The Firefly™ camera (Intuitive Surgical Inc., Sunnyvale, CA, USA) integrated into the da Vinci® robotic surgical system (Intuitive Surgical Inc., Sunnyvale, CA, USA) was used to image and record the fluorescence of anti-EGFR (199.12; Thermo Scientific, Rockford, IL, USA) conjugated to DyLight 800 in orthotopic tumors.

Tumor was resected after in vivo fluorescent imaging. Subsequently, hematoxylin and eosin staining of resected orthotopic tumors was performed and samples were observed under light microscopy

Declarations

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

J.H.C, C.M.K, and J.Y.P contributed to data acquisition and drafted the manuscript. C.M.K, and J.Y.P revised the manuscript. J.Y.P contributed to the study design. All authors read and approved the final manuscript.

Competing interests

J.H.C, C.M.K, and J.Y.P have no conflicts of interest or financial ties to disclose.

References

1. Ferlay, J. *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* **136**, E359-386 (2015).
2. Kim, B. W. *et al.* Incidence and Overall Survival of Biliary Tract Cancers in South Korea from 2006 to 2015: Using the National Health Information Database. *Gut Liver* **13**, 104-113 (2019).
3. Wi, Y., Woo, H., Won, Y. J., Jang, J. Y. & Shin, A. Trends in Gallbladder Cancer Incidence and Survival in Korea. *Cancer Res Treat* **50**, 1444-1451 (2018).
4. Hickman, L. & Contreras, C. Gallbladder Cancer: Diagnosis, Surgical Management, and Adjuvant Therapies. *Surg Clin North Am* **99**, 337-355 (2019).
5. Hiroshima, Y. *et al.* Successful fluorescence-guided surgery on human colon cancer patient-derived orthotopic xenograft mouse models using a fluorophore-conjugated anti-CEA antibody and a portable imaging system. *J Laparoendosc Adv Surg Tech A* **24**, 241-247 (2014).
6. Ahmad, A. Use of indocyanine green (ICG) augmented near-infrared fluorescence imaging in robotic radical resection of gallbladder adenocarcinomas. *Surg Endosc* **34**, 2490-2494 (2020).
7. van der Vorst, J. R. *et al.* Near-infrared fluorescence-guided resection of colorectal liver metastases. *Cancer* **119**, 3411-3418 (2013).
8. Yoon, Y. S. *et al.* Is Laparoscopy Contraindicated for Gallbladder Cancer? A 10-Year Prospective Cohort Study. *J Am Coll Surg* **221**, 847-853 (2015).
9. Shirobe, T. & Maruyama, S. Laparoscopic radical cholecystectomy with lymph node dissection for gallbladder carcinoma. *Surg Endosc* **29**, 2244-2250 (2015).
10. Shenoy, R. *et al.* Intraoperative and postoperative outcomes of robot-assisted cholecystectomy: a systematic review. *Syst Rev* **10**, 124 (2021).
11. Dip, F. D., Ishizawa, T., Kokudo, N. & Rosenthal, R. J. Fluorescence Imaging for Surgeons : Concepts and Applications *1st edn* 1 online resource (XXI, 360 pages 196 illustrations, 154 illustrations in color (Springer International Publishing : Imprint: Springer,, 2015).
12. Wendler, T., van Leeuwen, F. W. B., Navab, N. & van Oosterom, M. N. How molecular imaging will enable robotic precision surgery : The role of artificial intelligence, augmented reality, and navigation. *Eur J Nucl Med Mol Imaging* **48**, 4201-4224 (2021).
13. Hernot, S., van Manen, L., Debie, P., Mieog, J. S. D. & Vahrmeijer, A. L. Latest developments in molecular tracers for fluorescence image-guided cancer surgery. *Lancet Oncol* **20**, e354-e367 (2019).
14. Sigismund, S., Avanzato, D. & Lanzetti, L. Emerging functions of the EGFR in cancer. *Mol Oncol* **12**, 3-20 (2018).

15. Das, C., Mukhopadhyay, M., Subba, S., Saha, A. K. & Mukhopadhyay, B. Role of EGFR and HER-2/NEU Expression in Gall Bladder Carcinoma (GBC). *J Lab Physicians* **13**, 29-35 (2021).
16. Kaufman, M. *et al.* EGFR expression in gallbladder carcinoma in North America. *Int J Med Sci* **5**, 285-291 (2008).
17. Kang, C. M. *et al.* Laparoscopic cholecystectomy only could be an appropriate treatment for selected clinical R0 gallbladder carcinoma. *Surg Endosc* **21**, 1582-1587 (2007).
18. Kang, C. M. *et al.* Does "clinical" R0 have validity in the choice of simple cholecystectomy for gallbladder carcinoma? *J Gastrointest Surg* **11**, 1309-1316 (2007).
19. Lee, H. *et al.* Optimal extent of surgery for early gallbladder cancer with regard to long-term survival: a meta-analysis. *J Hepatobiliary Pancreat Sci* **25**, 131-141 (2018).
20. Kang, H. *et al.* Prognostic Significance of Tumor Location in T2 Gallbladder Cancer: A Systematic Review and Meta-Analysis. *J Clin Med* **10** (2021).
21. Lee, S. E. *et al.* Practical guidelines for the surgical treatment of gallbladder cancer. *J Korean Med Sci* **29**, 1333-1340 (2014).
22. Rosenthal, E. L. *et al.* Safety and Tumor Specificity of Cetuximab-IRDye800 for Surgical Navigation in Head and Neck Cancer. *Clin Cancer Res* **21**, 3658-3666 (2015).
23. Tummers, W. S. *et al.* Intraoperative Pancreatic Cancer Detection using Tumor-Specific Multimodality Molecular Imaging. *Ann Surg Oncol* **25**, 1880-1888 (2018).
24. Miller, S. E. *et al.* First-in-human intraoperative near-infrared fluorescence imaging of glioblastoma using cetuximab-IRDye800. *J Neurooncol* **139**, 135-143 (2018).
25. van Keulen, S. *et al.* Rapid, non-invasive fluorescence margin assessment: Optical specimen mapping in oral squamous cell carcinoma. *Oral Oncol* **88**, 58-65 (2019).
26. Zhou, Q. *et al.* EGFR-targeted intraoperative fluorescence imaging detects high-grade glioma with panitumumab-IRDye800 in a phase 1 clinical trial. *Theranostics* **11**, 7130-7143 (2021).
27. Marston, J. C. *et al.* Panitumumab-IRDye800CW for Fluorescence-Guided Surgical Resection of Colorectal Cancer. *J Surg Res* **239**, 44-51 (2019).
28. Doval, D. C., Azam, S., Sinha, R., Batra, U. & Mehta, A. Expression of epidermal growth factor receptor, p53, Bcl2, vascular endothelial growth factor, cyclooxygenase-2, cyclin D1, human epidermal receptor-2 and Ki-67: Association with clinicopathological profiles and outcomes in gallbladder carcinoma. *J Carcinog* **13**, 10 (2014).

29. Hoogstins, C. E. *et al.* A Novel Tumor-Specific Agent for Intraoperative Near-Infrared Fluorescence Imaging: A Translational Study in Healthy Volunteers and Patients with Ovarian Cancer. *Clin Cancer Res* **22**, 2929-2938 (2016).
30. Harlaar, N. J. *et al.* Molecular fluorescence-guided surgery of peritoneal carcinomatosis of colorectal origin: a single-centre feasibility study. *Lancet Gastroenterol Hepatol* **1**, 283-290 (2016).
31. Boogerd, L. S. F. *et al.* Safety and effectiveness of SGM-101, a fluorescent antibody targeting carcinoembryonic antigen, for intraoperative detection of colorectal cancer: a dose-escalation pilot study. *Lancet Gastroenterol Hepatol* **3**, 181-191 (2018).
32. Smith, B. L. *et al.* Real-time, intraoperative detection of residual breast cancer in lumpectomy cavity walls using a novel cathepsin-activated fluorescent imaging system. *Breast Cancer Res Treat* **171**, 413-420 (2018).
33. Unkart, J. T. *et al.* Intraoperative Tumor Detection Using a Ratiometric Activatable Fluorescent Peptide: A First-in-Human Phase 1 Study. *Ann Surg Oncol* **24**, 3167-3173 (2017).
34. Lee, N. P., Chan, C. M., Tung, L. N., Wang, H. K. & Law, S. Tumor xenograft animal models for esophageal squamous cell carcinoma. *J Biomed Sci* **25**, 66 (2018).
35. Mita, Y. *et al.* Antitumor effect of gemcitabine on orthotopically inoculated human gallbladder cancer cells in nude mice. *Ann Surg Oncol* **14**, 1374-1380 (2007).

Figures

Figure 1

Characterization of gallbladder tumor cell lines. (a) Western blot analysis of EGFR expression in NOZ cells (right lane), and in not HEK cells (left lane). (b) Flow cytometric analysis of fluorescent anti-EGFR bounding in NOZ cells (blue line) and HEK cells (red line). (c) Labeling of NOZ and HEK cells with fluorescent anti-EGFR. (x 40 water immersion objective with the LSM 700 confocal microscope, using 405- and 488-nm laser). The white scale bar represents 10 μ m.

Figure 2

Imaging of EGFR in subcutaneously-transplanted gallbladder tumors in vivo. (a) Representative images of NOZ and HEK cell tumors are shown. The mouse was imaged under both white light and fluorescence illumination. (IVIS, using a 650-nm laser) (b) NOZ and HEK cell tumors stained Hematoxylin and eosin staining (x 200) and fluorescent anti-EGFR (x 200, using 405- and 488- nm laser). The red scale bar represents 100 μ m.

Figure 3

Imaging of EGFR in orthotopically-transplanted NOZ cell gallbladder tumors with IVIS. (a) Fluorescence signals from orthotopically transplanted gallbladder tumors. (using a 650-nm laser) The red scale bar represents 1 cm. (b) Hematoxylin and eosin staining of orthotopically transplanted NOZ cell gallbladder tumors and normal liver tissues (x 200). The red scale bar represents 100 μ m.

Figure 4

Imaging of EGFR in orthotopically-transplanted NOZ gallbladder tumors using the Firefly camera. Fluorescence signals from gallbladder tumors orthotopically transplanted in the gallbladder were detected. (using an 800-nm laser). The red scale bar represents 1 cm.