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Near-infrared–conjugated humanized anti-carcinoembryonic antigen antibody targets colon cancer in an orthotopic nude-mouse model

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ABSTRACT

Background: The success of a curative surgery for cancer is dependent on the complete removal of all cancer cells. Tumor visualization by the surgeon can be enhanced through fluorescent-antibody targeting. To further develop such technology, we selected humanized anti-carcinoembryonic antigen (CEA) conjugated to a near-infrared dye to target orthotopically-implanted human colon cancer in nude mice.

Materials and methods: The HT-29 human colon cancer cell line was grown in culture and subcutaneously injected in mice. After 3 wk of growth, tumors were resected and cut into 2 mm^3 fragments that were sutured to the cecum of five additional nude mice for orthotopic implantation. The tumors were allowed to grow for 4 wk at which point 3 had successful orthotopic tumor growth and were selected for injection of the humanized anti-CEA antibody conjugated to the near-infrared dye IRDye800CW (anti-CEA-IRDye800CW). The antibody-dye conjugate (75 μg) was administered via tail vein injection. Images were obtained with the Pearl Trilogy Small Animal Imaging System with both 700 and 800 nm channels and evaluated using Image Studio.

Results: Laparotomy was performed 24 h after labeling the tumors. When imaged through the 800 nm channel, the tumors were observed to be strongly labeled with anti-CEA-IRDye800. At 48 h, laparotomy was repeated which again demonstrated strong labeling of the tumors through the 800 nm channel, but with a lower absolute intensity (in relative units), than at 24 h.

Conclusions: Humanized anti-CEA-IRDye800CW can rapidly and effectively label CEA-expressing human colon cancer in an orthotopic nude mouse model. Given the ability

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of this technology to target and label tumors with great specificity, the anti-CEA-IRDye800CW is currently being developed for clinical use in fluorescence-guided surgery.

Introduction

Fluorescence-guided surgery (FGS) combines advanced imaging platforms with targeted fluorescent agents to enhance neoplasms and improve their intraoperative detection by the surgeon.[1] One technique is to covalently bind an organic dye to a monoclonal antibody targeting a known tumor-specific antigen.[2] One such tumor biomarker that is being developed is anti-carcinoembryonic antigen (CEA) antibodies to target and label human colorectal cancer in nude mouse models.[3-6] Mouse and chimeric (mouse/human) antibodies against CEA have been conjugated with fluorescent dye and are capable of enhancing visualization of submillimeter tumor deposits[5] and successful FGS.[7,8] This technique has been applied to human clinical trials to localize squamous cell carcinoma of the head and neck using a cetuximab-IRDye800CW conjugate.[9]

In an effort to improve therapeutic efficacy in the clinic, Yazaki *et al*. humanized the murine anti-CEA T84.66 antibody with structurally similar "human" segments through a technique known as complementary-determining region grafting.[10] This "humanized" antibody, which is currently in clinical trials, was selected for the present study to determine the extent of fluorophore labeling of human colon cancer in an orthotopic mouse model. IRDye800CW was selected as the fluorophore for this study because of its preliminary success in human use. Furthermore, the dye has a similar excitation and emission profile to indocyanine green, for which numerous clinically available fluorescence imaging protocols and instrumentation already exist.[11,12]

Materials and methods

Cell culture

The human colon cancer cell line HT-29 was grown in Roswell Park Memorial Institute (RPMI) medium (Gibco-BRL, Grand Island, NY) supplemented with 10% fetal calf serum (Hyclone, Logan, UT) and 1% penicillin–streptomycin (Gibco-BRL). The cells were cultured at 37°C in a 5% CO_2 incubator.

Conjugation of antibody to fluorophore

The humanized monoclonal antibody hT84.55-M5A specific for CEA was conjugated with NHS-IRDye800CW (generous gift from LI-COR Biosciences, Lincoln, NE). Briefly, the antibody was combined with reconstituted reactive dye at a molar ratio of 10:1 (dye:antibody) in 0.1 M sodium bicarbonate and allowed to incubate at room temperature for 1 h then overnight at 4°C. Excess dye was removed through an Amicon stirred cell concentrator (Millipore, Billerica, MA). The final concentration of antibody-dye conjugate was 6.6 mg/mL with an average of 1.6 dye molecules per immunoglobulin G. The antibody-dye conjugate was stored in the 4°C refrigerator and was protected from light.

Animal care

Athymic *nu/nu* nude mice (AntiCancer, Inc, San Diego, CA), between 4 and 6 wk of age, were maintained in a barrier facility at AntiCancer, Inc, on high-efficiency particulate air-filtered racks. The animals were fed with autoclaved laboratory rodent diet (Teckland LM-485; Western Research Products, Orange, CA). All surgical procedures and imaging were performed with the animals anesthetized by intramuscular injection of 0.02 mL of a solution of 50% ketamine, 38% xylazine, and 12% acepromazine maleate. All animal studies were conducted in accordance with the principles and procedures outlined in the NIH Guide for the Care and Use of Animals under Public Health Service license number A3873-1.

Subcutaneous injection of cancer cells

The HT-29 line of human colorectal cancer cells were harvested after 2 wk of growth by trypsinization and washed with serum-free medium. Cells (1×10^6) were combined with 100 μL Matrigel (Corning, Tewksbury, MA) and injected into the bilateral flanks of five athymic female *nu/nu* mice at 6 wk of age. The tumors were allowed to grow until they reached a diameter of approximately 10 mm, which occurred after 3 wk.

Passage and orthotopic implantation of HT-29 tumor

Orthotopic human colon cancer xenografts were established in nude mice by suturing a small fragment of HT-29 tumor on the mesenteric border of the mouse cecum. To start, a 10 mm subcutaneous HT-29 tumor was resected and cut into 2 mm^3 fragments. The fragments were then sutured to the cecum of five additional nude mice using 8-0 nylon sutures. The tumors were allowed to grow for 4 wk at which point 3 had successful orthotopic tumor growth and were selected for fluorescence imaging. The remaining two mice did not survive the 4-wk recovery period. Small tumor fragments were also implanted in the bilateral flanks of each mouse to track the growth of the tumors.

Antibody-dye conjugate delivery

Four weeks after the orthotopic implantation of HT-29 tumor, the animals were given a single intravenous dose of 100 μL (75 μg) of anti-CEA-IRDye800CW via tail vein injection.

Mouse imaging

Images were obtained with the Pearl Trilogy Small Animal Imaging System (LI-COR) 24 and 48 h postinjection (with laparotomy views) with both 700 and 800 nm channels. Images