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Development of a metastatic murine colon cancer model

Krista P. Terracina, MD,[a] Tomoyoshi Aoyagi, MD, PhD,[a,b] Wei-Ching Huang, PhD,[a,b] Masayuki Nagahashi, MD, PhD,[a,b] Akimitsu Yamada, MD,[a,b] Kazunori Aoki, MD,[c] and Kazuaki Takabe, MD, PhD, FACS[a,b,*]

[a] Division of Surgical Oncology, Department of Surgery, Virginia Commonwealth University School of Medicine and Massey Cancer Center, Richmond, Virginia

[b] Department of Biochemistry and Molecular Biology, Virginia Commonwealth University School of Medicine, Richmond, Virginia

[c] Division of Gene and Immune Medicine, National Cancer Center Research Institute, Tokyo, Japan

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ABSTRACT

Background: It has now become clear that the complex interplay of cancer and the immune responses against it plays a critical role in the tumor microenvironment during cancer progression. As new targets for cancer treatment are being discovered and investigated, murine models used for preclinical studies need to include intact immune responses to provide a closer correlation with human cancer. We have recently developed a modified syngeneic orthotopic murine colon cancer model that mimics human colon cancer progression with consistent results.

Materials and methods: Tumors were created using the murine colon adenocarcinoma cell line, CT26, modified to overexpress the firefly luciferase gene (CT26-luc1), which allowed real-time *in vivo* monitoring of tumor burden when the substrate, D-luciferin, was injected intraperitoneally using the *In Vivo* Imaging System. Mice are Balb/c (Harlan), syngeneic with the CT26-luc1 cells. Cells are injected submucosally, suspended in Matrigel, into the cecum wall under direct visualization.

Results: The model has demonstrated consistent implantation in the cecum. *In vivo* bioluminescence allowed real-time monitoring of total tumor burden. Perioperative preparation had a significant impact on reproducibility of the model. Finally, total tumor burden quantified with bioluminescence enabled estimation of lymph node metastasis *ex vivo*.

Conclusions: This method maintains an intact immune response and closely approximates the clinical tumor microenvironment. It is expected to provide an invaluable murine metastatic colon cancer model particularly in preclinical studies for drug development targeting those mechanisms.

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* *Corresponding author*. Division of Surgical Oncology, Department of Surgery, Virginia Commonwealth University School of Medicine and Massey Cancer Center, PO Box 980011, West Hospital 7-402, 1200 East Broad Street, Richmond, VA 23298 0011. Tel.: +1 804 828 9322; fax: +1 804 828 4808.

E-mail address: ktakabe@vcu.edu (K. Takabe).

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1. Introduction

Colorectal cancer (CRC) is the third most common cause of death by cancer in both sexes in the United States, and over 50,000 are expected to succumb to CRC in 2013 [1]. With best care, the 5-y survival rate of all cases of CRC is 65%. It is clear that new therapies are still needed to improve survival in advanced disease. Although some promising therapies have been developed in recent years, the vast majority of compounds validated by animal models fail to show benefit in clinical trials. For drugs tested from 1993–2002, only 26% of oncology drugs tested in phase I trials resulted in Food and Drug Administration approval for treatment [2]. In recent years, a number of voices have brought awareness to the growing concern that our preclinical animal models are inappropriate for assessing the utility of novel therapies in actual human patients [3–5]. Criticisms point out the poor predictive value of many preclinical trials, the lack of relevance of *in vitro* data, and the differences between mouse models and human immunology and cancer biology.

Despite these criticisms, murine models for human cancer are still the mainstay of preclinical evaluation in drug discovery and emerging technologies. Thus, models that more closely replicate the biology and progression of human cancer are in urgent need. Xenograft models that implant human colon cancer cells into immune-deficient nude mice have been used since the 1960s [6] and remain the most commonly used model for drug development. Xenograft models are useful for assessing drug effectiveness against human cancer cells in an animal setting; however, as targeted therapies emerge, the immune-deficient nude mouse model is no longer an ideal model for novel therapies that take advantage of the immunologic characteristics of cancer. Even the latest patient-derived xenograft models that maintain the heterogeneity of the tumor by transplanting a part of a tumor from a patient, argued by many to be the best mimic of a human tumor, are not free from this limitation because the tumor can only be implanted onto immune-deficient nude mice [7].

Although syngeneic models that implant murine cells into immune intact normal mice are limited to using relatively less studied murine colon adenocarcinoma cell lines, these models are capable of demonstrating the complex interaction between the immune system and the tumor microenvironment in cancer progression. Subcutaneous tumors were for many years the mainstay of murine tumor models, with direct measurement of tumor size used to study effects of drugs and targeted therapies. We have recently reported that even these genetically homogenous cell lines have markedly different gene expression depending on the location of the tumor in the mouse, with subcutaneous tumors having a different gene expression profile from orthotopically implanted tumors, and models of metastasis, demonstrating the importance of orthotopic models in studying the effects of the tumor microenvironment on cancer growth and progression [8–10].

Real-time monitoring of cancer progression *in vivo* is now possible through genetic overexpression of reporter genes, such as green fluorescent protein (GFP) or luciferase, within the implanted cell lines. Methods using GFP were the first to be developed [11]; however, the strength of the signal is known to dissipate when it travels through the body wall. Syngeneic cancer cell lines engineered to express firefly luciferase have been used successfully in our laboratory to track breast cancer progression and metastasis to the lymph nodes and lungs [10,12] and are optimally suited for following intra-abdominal tumors and subsequent metastasis. Here, we demonstrate our newly established syngeneic colon cancer cell implantation method and the utility of our luciferase positive model for monitoring cancer progression.

2. Material and methods

2.1. *Cell culture*

CT26-luc1 cells generated from CT26 (American Type Culture Collection, Rockville, MD), a murine colon adenocarcinoma cell line derived from BALB/c colon, have been engineered to express firefly luciferase through a retrovirus vector-mediated process by our collaborators at the National Cancer Research Institute in Tokyo, Japan [13]. CT26-luc1 cells were cultured in RPMI Medium 1640 (Life technologies, Carlsbad, CA) with 10% fetal bovine serum. Before implantation, CT26-luc1 cells were cultured in a 37°C humidified incubator with 5% CO_2 and grown to 80% confluence using RPMI with 10% fetal bovine serum. Cells were resuspended in phosphate-buffered saline (PBS) and mixed with Matrigel Basement Membrane Matrix (BD Bioscience, San Jose, CA) at a ratio of 1:9 for a final concentration of 50,000/10 μL before implantation.

2.2. *Animals*

All animal studies were conducted in the Animal Research Core Facility at Virginia Commonwealth University School of Medicine in accordance with institutional guidelines. Surgical procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Female BALB/c mice (8–10 wk, weight 20–25 g; Harlan, Indianapolis, IN) were anesthetized with continuous vaporized isoflurane for general anesthesia, and mice were given analgesia (Buprenorphine SR, Zoopharm, Windsor, CO) for at least 72 h postoperatively and closely monitored throughout the perioperative period. Any animals appearing to be in significant distress or showing physical signs indicating unlikely survival for an additional 24 h were euthanized as a humane end point.

2.3. In vivo *bioluminescence*

D-Luciferin (0.2 mL of 15 mg/mL stock; PerkinElmer, Waltham, MA) was injected intraperitoneally into mice previously implanted with CT26-luc1 cells at indicated times. Bioluminescence was detected and measured using the *In Vivo* Imaging System (IVIS; Caliper–PerkinElmer). Living Image Software (Xenogen–PerkinElmer) was used to quantify the photons per second emitted by the cells. Bioluminescence was measured and quantified at 5-min intervals over 30 min using a subject