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Modified breast cancer model for preclinical immunotherapy studies

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ABSTRACT

Background: Interest in immunotherapy for breast cancer is rapidly emerging, and applicable animal models that mimic human cancer are urgently needed for preclinical studies. This study aimed to improve a technique for orthotopic inoculation of syngeneic breast cancer cells to be used as a preclinical animal model for immunotherapy.

Materials and methods: We used our previously reported murine model of orthotopic cancer cell inoculation under direct vision and compared the efficiency of tumorigenesis with tumor cells suspended in either phosphate-buffered saline or Matrigel containing varying numbers of cells. As a model for immune rejection, murine BALB/c–derived 4T1-*luc2* breast cancer cells were inoculated orthotopically into both BALB/c and C57BL/6 mice.

Results: Matrigel-suspended cells formed larger tumors with higher efficiency than phosphate-buffered saline-suspended cells. The maximum volume of Matrigel that could be inoculated without spillage was 20 μL and 30 μL in the #2 and #4 mammary fat pads, respectively. Tumor take rates increased as the injected cell number increased. In this immune rejection model, there were no significant differences in tumor weight between the strains up to day 7, after which tumor weight decreased in C57BL/6 mice. Bioluminescence in C57BL/6 mice was also significantly less than that in BALB/c mice and increased up to day 7, then swiftly decreased thereafter.

Conclusions: This improved technique of innoculating murine breast cancer cells using bioluminescence technology may be useful in evaluating the efficacy of tumor regression mediated by immune responses, as shown by an allogeneic response in C57BL/6 mice.

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Introduction

Immunotherapy, including checkpoint inhibitors, cancer vaccines, adoptive cell immunotherapy, and strategies that exploit chimeric antigen receptor–engineered T cells, is rapidly emerging as a promising modality for different types of cancers.[1] The early success of immune checkpoint inhibition, such as targeted therapy against cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), along with programmed death-1 (PD-1)[2,3] and the PD-1 ligand, PD-L1,[4] has drawn much attention.[5]

One of the most commonly used animal models for oncologic preclinical studies is xenografts of human cancer cells or patient-derived cancer tissue into immune-deficient mice.[6,7] However, these models are not suitable to evaluate immune responses or the effects of immunotherapy because the host animal lacks a fully functioning immune system. Spontaneous tumorigenic models using transgenic mice or carcinogens have been developed in animals with an intact immune system, but these models may require a long waiting time for the development of cancer, which limits their practicality.[8,9] Furthermore, these models usually require expensive equipment, such as mouse-specific imaging scanners (computed tomography, magnetic resonance imaging, or positron emission tomography), to detect and measure tumors. Even with such state-of-the-art diagnostic imaging equipment, the evaluation of immunotherapy responses remains challenging. Tumor size may not necessarily reflect the amount of cancer cells, as tumors may initially enlarge on imaging studies due to accumulated infiltrating immune cells when immunotherapy is actually effective. Recently, patient-derived "humanized" xenograft models have been developed using patient tumors implanted into immune-deficient mice that are engineered to have intact human immune cells.[10-12] However, the cost of these animals and other limitations, including take rate, viral contamination and selection pressure, hinder this approach,[10] and even these modern models cannot escape from the challenges of assessing tumor responses to immunotherapy.[12] Thus, orthotopic inoculation of syngeneic murine tumor cells tagged with a bioluminescent reporter into immune intact mice is the most straightforward, fast, and affordable model to study the effect of immunotherapy at this point.

We have previously reported the establishment of a murine syngeneic breast cancer model using cell inoculation into chest mammary fat pads under direct vision, which can mimic human cancer progression.[13-15] In this study, we report the establishment of an improved orthotopic inoculation technique of murine breast cancer cells using *luciferase*-tagged 4T1-*luc2* murine cancer cells suspended in Matrigel and demonstrate that this model is useful to assess immune-mediated regression of breast tumors.

Materials and methods

Cell culture

4T1-*luc2* cells, a mouse mammary adenocarcinoma cell line derived from BALB/c mice that has been engineered to express luciferase was purchased from Caliper Life Sciences/PerkinElmer (Hopkinton, MA). 4T1-*luc2* cells were cultured in RPMI Medium 1640 with 10% fetal bovine serum. E0771 cells, a C57BL/6 mouse mammary fat pad–derived adenocarcinoma cell line, were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum.

Animal models

Approval from the Virginia Commonwealth University Institutional Animal Care and Use Committee was obtained for all experiments. Female BALB/c and C57BL/6 mice were obtained from Jackson Laboratory. All cell inoculations into #2 (chest) and #4 (abdomen) mammary fat pads under direct vision were carried out as previously described.[13] All procedures were performed using sterile technique under isoflurane anesthesia, and the animals were prepped and draped in a sterile manner. In brief, a 10-mm incision was made medial to the nipple, and a cotton swab was used to expose the mammary gland. A syringe with a 26-G needle was used to inject the cell suspension directly into the mammary gland under direct vision, and the wound was closed with a suture. To maximize the take rate, cell inoculations were conducted within 1 h from preparation of cell suspensions. Tumor growth was monitored by caliper measurement, and animals were weighed every other day.

Preparation of Matrigel and phosphate-buffered saline cell suspensions

1×10^4 of 4T1-*luc2* cells were suspended in 20 μL of either phosphate-buffered saline (PBS) or Matrigel. Cells were inoculated into #2 and #4 mammary fat pads of BALB/c mice ($n = 8$). Fourteen days after inoculation, tumors were assessed by palpation, then harvested, and weighed.

Determination of the optimum amount of Matrigel for injection into mammary fat pads

To determine the amount of Matrigel a mammary fat pad can hold, 10 μL of Matrigel stained with 10% Trypan blue was injected incrementally into the #2 and #4 mammary fat pads ($n = 12$, each group). Spillage of Matrigel out of the fat pad was assessed visually after each injection.

Tumorigenesis with different numbers of cells inoculated

4T1-luc2 cells (5×10^2, 1×10^3, 5×10^3, 1×10^4) or E0771 cells (5×10^4, 1×10^5, 5×10^5, 1×10^6) were suspended in 20 μL of Matrigel, then inoculated into the #2 and #4 mammary fat pads of C57BL/6 or BALB/c mice, respectively ($n = 8$, each group). Formation of tumors was determined by palpation. Larger numbers of E0771 cells were inoculated because of the slower growth rate of this tumor in the appropriate syngeneic mouse strain.

4T1-luc2 tumors in C57BL/6 mice (immune rejection model)

1×10^4 of 4T1-*luc2* cells, derived from BALB/c mice, suspended in Matrigel were implanted into the right #2 fat pad of C57BL/6 mice or BALB/c mice as a control. Tumor growth was monitored every other day by bioluminescence (IVIS)