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Murine breast cancer mastectomy model that predicts patient outcomes for drug development

Eriko Katsuta, MD, PhD,[a] *Omar M. Rashid, MD, JD,*[b,c,d,e] *and Kazuaki Takabe, MD, PhD, FACS*[a,f,*]

[a] *Breast Surgery, Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, New York*
[b] *Holy Cross Hospital Michael and Dianne Bienes Comprehensive Cancer Center, Fort Lauderdale, Florida*
[c] *Department of Surgery, Massachusetts General Hospital, Boston, Massachusetts*
[d] *Department of Surgery, University of Miami Miller School of Medicine, Miami, Florida*
[e] *Department of Surgery, Nova Southeastern University School of Medicine, Fort Lauderdale, Florida*
[f] *Department of Surgery, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, The State University of New York, Buffalo, New York*

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ABSTRACT

Background: Despite massive expenditures in preclinical studies, many breast cancer agents show efficacy in murine models but fail in human trials. In humans, metastatic disease determines survival, but preclinical murine models only evaluate drug efficacy against the primary tumor. We hypothesized that evaluating efficacy against metastatic breast cancer would more efficiently predict efficacy in a murine model than evaluating the primary tumor alone. This study (1) critically evaluated a murine tumor removal model with metastatic tumor burden quantification for breast cancer preclinical trials and (2) validated the model with an agent that previously passed preclinical trials but failed human trials.

Materials and methods: Tumorectomy and Halsted (radical) mastectomy procedures after inoculation of 4T1-*luc2* cells were compared. The effect of AZD0530, an oral Src inhibitor that passed preclinical trials but failed human trials, was evaluated using an inoculation model with/without Halsted mastectomy.

Results: Significant amounts of residual disease were confirmed by bioluminescence ($P = 0.003$) and 100% developed local recurrence after tumorectomy *versus* 14% ($P = 0.005$) after Halsted mastectomy. Bioluminescence value at 15 min after luciferin injection highly correlated with peak except for 24 h after injection. AZD0530 significantly suppressed primary tumor burden compared with no treatment ($P = 0.002$); but not in lung metastases. In a Halsted mastectomy model, AZD0530 had no efficacy against lung metastases or difference in survival.

Conclusions: We critically evaluated and established a murine mastectomy model to evaluate metastatic tumors. It provides a new model for preclinical drug development that mimics the human adjuvant setting.

* *Corresponding author*. Breast Surgery, Department of Surgical Oncology, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263, USA. Tel.: +1 716 845 5128; fax: +1 716 845 5705.
E-mail address: kazuaki.takabe@roswellpark.org (K. Takabe).
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Introduction

Breast cancer is the most prevalent and second leading cause of cancer-related death in women in the US.[1] Although billions of US dollars have been invested in treating breast cancer, large portions of these investments have been lost in efforts that have failed to deliver.[2,3] Given the huge cost required for human clinical trials that a drug needs to pass to prove its efficacy for patients, it is conducted only for drugs that show significant promise in preclinical animal experiments.[2] However, despite these efforts, most drugs that passed murine studies still failed in human clinical trials,[4] which at least partly can be explained by the use of murine models that do not represent human conditions.[2] To predict the outcome of human clinical trials, murine models for preclinical study need to reproduce the condition of human cancer.[2,3,5,6]

To date, the most commonly used murine breast cancer models evaluate drug efficacy against primary tumors, either in orthotopic (mammary pads) or ectopic sites (subcutaneous tissues).[2,3,5,6] Although drugs are sometimes used in humans as a neoadjuvant therapy to treat the primary breast tumor, the vast majority is used as adjuvant therapy where drugs are given after surgery to reduce the risk of recurrence by treating undetectable remaining or metastatic cancer.[2,3,5] Recently, our group and others have shown that the genetic profiles of metastatic lung tumors are significantly different from that of their primary tumors, which implicates that their biology is different.[2,3,7,8] Therefore, we hypothesized that metastatic tumors may respond to drugs differently from primary tumors. Accordingly, a new drug should be evaluated by its efficacy against metastatic tumors. To our knowledge, there are few reports utilizing murine tumor removal models for preclinical drug development studies,[2,3,5,6] although we have previously reported the use of a murine mastectomy model in breast cancer nondrug development research.[6,9]

Because of the disconnect between what determines breast cancer survival in humans and how murine models determine drug efficacy, we hypothesized that aligning murine models more with the human condition would improve the efficiency of breast cancer drug development. Accordingly, we hypothesized that a model that evaluates efficacy against metastatic breast cancer would more efficiently predict efficacy in humans than one that evaluates efficacy against the primary tumor alone. Therefore, the study (1) critically evaluated a murine radical mastectomy (Halsted) model with metastatic tumor burden quantification for breast cancer preclinical trials and (2) validated the model with an agent that previously passed preclinical trials but failed human trials.

Material 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, were used (Caliper Life Sciences/PerkinElmer, Hopkinton, MA). 4T1-*luc2* cells were cultured in RPMI 1640 Medium with 10% fetal bovine serum.

Animal models

Approval from the Roswell Park Cancer Institute Institutional Animal Care and Use Committee was obtained for all experiments. Female BALB/c mice aged 9-12 wk were obtained from Jackson Laboratory. 1×10^5 4T1-*luc2* cells in 20-μL Matrigel were inoculated into #2 fat pads under direct vision as previously described.[4-6,10] Eight days after inoculation, either tumorectomy or Halsted mastectomy were performed ($n = 7$, each). Resected tumor weight, bioluminescence on the day before tumor resection and on the day after tumor removal, and recurrence rate were compared between these two procedures using previously reported techniques.[4,6,10-13]

Tumorectomy

Spindle-shaped skin incision was made to remove the surgical scar, which was made at inoculation. The skin was then inverted, and the tumor was resected off the chest wall. After the tumor was completely freed from the chest wall, the tumor was also resected from the skin and the wound was closed with suture (Fig. 1).

Halsted (radical) mastectomy

William Stewart Halsted M.D., who established surgical residency training and is often named as the greatest surgeon in American surgery, established the operation radical mastectomy, removal of all the breast tissue with the skin, including the nipple-areola complex, axillary lymph nodes, and pectoralis major muscle. Honoring his contribution to surgical research, we named our mouse model Halsted mastectomy, which removes all the structures named above. Briefly, skin incision was made 5 mm from the inoculation surgical scar. Then, it was extended toward both cephalad and caudad to include both the scar and tumor. The tumor was removed from the chest wall *en bloc* with the pectoralis muscle, and the skin incision was extended to the axilla to anatomically allow for standard axillary lymph node dissection (only swollen lymph nodes were visible). The wound was closed with suture (Fig. 2). After removal, only the primary tumor was detached from surrounding tissue including, fat, muscle, lymph nodes, and skin and weighed for tumor weight comparison.

Treatment with AZD0530 with or without mastectomy

1×10^5 and 1×10^4 4T1-*luc2* cells were inoculated for with and without mastectomy in BALB/c mice. A smaller number of cells were inoculated for the orthotopic model because 4T1 primary tumors grow too fast and quickly reach euthanasia criteria. On the other hand, a larger number of cells were inoculated for mastectomy model because mastectomy needs to be conducted after metastatic cells spread. Halsted mastectomy was performed on 8 days after inoculation of cancer cells. AZD0530 was administrated by oral