BIOLOMINEUSCENT ANIMAL MODELS OF HUMAN BREAST CANCER FOR TUMOR BIOMASS EVALUATION AND METASTASIS DETECTION

Liang Shan, MD, PhD; Songping Wang, PhD; Alexandru Korotcov, PhD; Rajagopalan Sridhar, PhD; Paul C. Wang, PhD

INTRODUCTION

Biomimetic imaging (BLI) is an imaging modality that enables rapid in vivo analyses of a variety of cellular and molecular events with extreme sensitivity. This imaging technique is based on light-emitting enzymes, such as luciferase, as internal biological light sources that can be detected externally as biological indicators. As a result of recent developments in techniques for high-sensitivity detection of bioluminescence, BLI has been recently tested in the detection and real-time observation of primary tumor growth and metastasis in living subjects. Luciferase-based light-emitting animal models have also been used to develop therapeutics that target the molecular basis of disease. Importantly, BLI provides a biosystem to test the spatial-temporal expression patterns of both target and therapeutic genes in living animals where the contextual influences of whole biological systems are intact. In this study, we established three bioluminescent animal models of human breast cancer using MDA-MB-231-luc cell line, which has been stably transfected with the luciferase gene. The primary and metastatic lesions were analyzed through whole-animal imaging, and the tumor volume was evaluated in relationship with the bioluminescent signal intensity.

METHODS

Cell culture and animal models

MDA-MB-231-luc human breast cancer cell line and D-luciferin were obtained from Xenogen (Alameda, Calif). This cell line has been stably transfected with luciferase gene for luciferase-based BLI. Cells were routinely maintained in Dulbecco minimal essential medium/F-12 medium supplemented with 10% heat inactivated fetal bovine serum and 50 μg/mL each penicillin, streptomycin, and neomycin (Invitrogen, Carlsbad, Calif). Female athymic nude mice of 8–10 weeks of age (n=16) were purchased from Harlan (Indianapolis, Ind). Three animal models were developed. The subcutaneous solid tumor xenograft model was developed by subcutaneous injection of 1×10⁶ subconfluent cells in 100 µL Dulbecco phosphate buffered saline (DPBS) in the right lower back of each mouse (n=8). The mammary gland fat pad tumor model was developed by injection of 1×10⁷ subconfluent cells in 100 µL DPBS into the right fifth mammary gland fat pad (n=5). Matrigel or other anchoring matrix was not used to produce the tumors. The lung metastasis model of breast cancer was developed by tail vein injection of 1×10⁶ tumor cells (n=3). The tumors in subcutaneous tissue and mammary gland fat pad were imaged and analyzed when they reached a certain size (3–11 mm diameter). For lung metastatic model, whole animals were checked weekly and autopsied when tumor signal from the lung region was detected.

In vivo BLI

Luciferase-based BLI was performed with a highly sensitive, cooled charge-coupled device camera mounted in a light-tight specimen box (Xenogen IVIS 200 imaging system). Imaging and quantification of signals were controlled by the acquisition and analysis software Living Image (Xenogen). Mice were placed onto the warmed stage inside the light-tight camera box with continuous exposure to 2% isofluurane. After a...