Abstract

Breast carcinoma is one of major cancer mortality in women, and the metastasis of this disease is the leading cause of cancer-related deaths in patients. According to the 2004 Annual Report of the Department of Health, Executive Council of Taiwan, breast cancer is ranked 4th of the major cancer incidence in both women and population. The metastatic process is a series of interactions among cancerous cells and host cells or tissues.

Developing optimal animal models that more closely simulate human diseases using immunodeficient mice has been shown to be beneficial for experimental analysis of human breast cancer. The studies of breast cancer metastasis have been dominated by two human breast cancer cell lines, MDA-MB-435 and MDA-MB-231. In this study, we generated MDA-MB-435s-thorax human breast carcinoma xenografts that would provide tumor growth kinetics, produce metastatic spreading, and permit in vivo detection over times using multimodalities of various molecular imaging approaches.

Materials and Methods

The results demonstrated that MDA-MB-435s-thorax orthotopic tumors in mice could be visualized repeatedly and non-invasively with bioluminescence imaging (BLI). Sequential scanning of mice allowed the kinetics of tumor growth to be monitored and quantified. Following intravenous injection of the MDA-MB-435s-thorax tumors, early metastasis to axial skeleton, lung, lymph nodes, adrenal gland and various visceral organs was detected.

Introduction

Conventional methods to assay tumor growth and metastasis in murine models require time-consuming histological examinations or molecular tests on selected tissue samples obtained either from biopsy or autopsies rather than a whole body analysis. In the past few years, non-invasive imaging of receptor expression can be performed within a single cell to groups of cells within a living subject using various imaging modalities, including positron emission tomography (PET), single photon emission tomography (SPECT), and optical imaging, is playing an increasing important role in understanding normal physiology and disease progression.

Luciferase simplex virus type 1 thymidine kinase (HSV1-1k) and nuclear medicine imaging

Inside the transfected cell, the HSV1-1k gene is transcribed and then translated into an enzyme, HSV1-TK. After administration of a radiolabeled probe and transport into the cell, the probe is phosphorylated by HSV1-TK. The phosphorylated radiolabeled probe does not readily cross the cell membrane and is trapped within the cell.

Luciferase (luc) and bioluminescent imaging (BLI)

“Luciferase” is a family of protein-photons that can be isolated from a wide variety of insects, marine organisms, and prokaryotes. Luciferase could react with the substrates “sulfuric”, resulting in the formation of a luciferase bound peroxy-luciferin intermediate, which releases photons of visible light.

Results


Table 1. Bioluminescence Imaging in Various Tissues/Organs

<table>
<thead>
<tr>
<th>Tissue/Organ</th>
<th>ROI 1 (counts/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1.2665e+08</td>
</tr>
<tr>
<td>Lung</td>
<td>1.0156e+06</td>
</tr>
<tr>
<td>Left ribs</td>
<td>7.3549e+05</td>
</tr>
<tr>
<td>Right ribs</td>
<td>1.8094e+06</td>
</tr>
</tbody>
</table>

Figure 1. Monitoring the bioluminescence and growth of orthotopic tumors.

Figure 2. Whole-body autoradiography (WBAR) confirmed metastatic lesions detected by BLI. (A) Bioluminescent signal was detected in the thorax of mouse via intravenous injection of 1 × 10^6 MDA-MB-435s-thorax cells. Following non-invasive and invasive imaging was applied to validate the tumor metastasis. (B) After injection of 200 μCi ⁴⁷⁰⁰⁻FIAU, mouse was scanned with digital gamma camera. The whole body image was acquired by a high sensitivity gamma camera. The figure demonstrated that there was no obvious uptake of ⁴⁷⁰⁰⁻FIAU in the thorax because of low sensitivity and spatial resolution. The mouse was sacrificed immediately after scanning, and WBAR was carried out. (C) Digital photo showed the detailed anatomy. (D) Autoradiogram clearly showing trapping of radiotracer in the lung, and the lung-to-brain ratio was 15-fold higher than control group (23.51±0.97 vs 1.53±0.04, respectively). BLI confirmed the presence of tumor nod in lung, consistent with the BLI.

Figure 3. Whole-body autoradiography (WBAR) confirmed metastatic lesions detected by BLI. (A) Bioluminescent signal was detected in the thorax of mouse via intravenous injection of 1 × 10^6 MDA-MB-435s-thorax cells. Following non-invasive and invasive imaging was applied to validate the tumor metastasis. (B) After injection of 200 μCi ⁴⁷⁰⁰⁻FIAU, mouse was scanned with digital gamma camera. The whole body image was acquired by a high sensitivity gamma camera. The figure demonstrated that there was no obvious uptake of ⁴⁷⁰⁰⁻FIAU in the thorax because of low sensitivity and spatial resolution. The mouse was sacrificed immediately after scanning, and WBAR was carried out. (C) Digital photo showed the detailed anatomy. (D) Autoradiogram clearly showing trapping of radiotracer in the lung, and the lung-to-brain ratio was 15-fold higher than control group (23.51±0.97 vs 1.53±0.04, respectively). BLI confirmed the presence of tumor nod in lung, consistent with the BLI.

Conclusions

Continuous scanning of tumor burden over times provides a more realistic kinetics of the tumorigenesis and treatment.

The advantages of in vivo imaging to determine the distribution of metastasis is that these images readily capture quantitative and qualitative information from multiple tissues simultaneously.

BLI is much more sensitive for early detection of metastatic tumors, when gamma scintigraphy and microPET are incapable to do so.

Like in human, animal models with MDA-MB-435s-thorax tumors have high potential to develop skeletal metastasis. In addition, tumors also colonize in several visceral organs and soft tissues.

Dual reporter genes (HSV1-TK/luc) conjugated with multimodalities of molecular imaging can facilitate studies on the molecular mechanisms of human breast cancer.

MDA-MB-435s-thorax animal model also offers rapid and highly sensitive assessment for disease progression as well as for anticancer therapy in preclinical studies.