Non-invasive Detection of Bone Metastases using Co-registration of Optical Imaging with MicroCT

Angel Ang, Konnie Urban, Chaincy Kuo, Kevin Francil, Raj Singh, Mark Roskey and Jae-Beom Kim, Caliper Life Sciences, Alameda, CA USA

Abstract

We previously engineered tumor cell line with bioluminescent markers and fluorescent protein for whole animal imaging. In addition to conventional non-invasive tumor growth detection, these cells when harvested from animals can also be used to analyze cell-cell interactions by immunohistochemistry. Moreover, fluorescent cells can be isolated using fluorescent activated cell sorting (FACS) for gene expression profiling. In this study, we used a mouse mammary gland tumor cell line 4T1 that was labeled with firefly luciferase and tdTomato fluorescent protein (4T1-luc2-tdTomato).

The generated cell line was tested for in vivo signal reliability with respect to bioluminescence and fluorescence prior to injection into the animals. Tumor cells were implanted subcutaneously and monitored for tumor development by acquisition of bioluminescent and fluorescent images. In a separate study 4T1-luc2-tdTomato cells were implanted orthotopically into mammary fat pads. Tumor growth was monitored and signals from the implantation sites were reconstructed into three dimensional images. In order to non-invasively detect bone metastases by 4T1-luc2-tdTomato cells, we applied both microCT and optical imaging. For this, an interchangeable imaging platform between an optical and microCT scanner was carried on to locate bioluminescent signals on the joint region. For these animals, bioluminescent and Xray images were also taken without changing the positions of the animals using the interchangeable platform. Bioluminescent images in the joint regions were reconstructed using a diffused luminescence imaging tomography algorithms. These images were then co-registered with microCT images. The results showed that bioluminescent signals were colocalized within the joint area obtained by microCT. Moreover, high resolution images of the joints from these mice revealed bone erosion in the tibia induced by 4T1-luc2-tdTomato cells. These findings demonstrate that multimodal imaging can pinpoint the tumor location non-invasively. In addition, low dose microCT imaging can be used to acquire high resolution skeletal images longitudinally without inducing adverse effects on the animals.

Materials and Methods

Generation of Fluorescent Protein Expression Vector

Enhanced luciferase 2 (luc2) DNA was from pGL2-20 vector (Promega, WI). Luciferase 2 expression vector was previously reported[1]. tdTomato DNA was cloned into pTRE2-hygro (Clontech). A fragment generated by BglII and XbaI digestion of pTRE2-hygro was ligated into the modified pTRE2 vector (pTRE2-TdTomato, Sigma-Aldrich, MO).

Cell Culture

Tumor cell line 4T1 was obtained from ATCC (Manassas, VA). Cells were grown in high-glucose RPMI 1640 medium (ATCC) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) without antibiotics.

Transfection

Dual labeled cells were generated by transfecting pTRE2-TdTomato into parental luc2 cell line 4T1-luc2 (4T1-luc2-tdTomato). Transfected cells were selected using puromycin. Isolated clones were screened for their luciferase and fluorescent protein activities using an in vivo imaging system equipped with an IVIS Spectrum (Caliper Life Sciences, Hopkinton, MA). Cells expressing both proteins were sorted on a FACSort imaging flow cytometer equipped with a green laser (Beckton Dickinson). The gating strategy included three separate gates: in the first gate, tdTomato and luciferase 2 positive cells were selected; in the second gate, only luciferase 2 positive cells were selected and in the third gate, only tdTomato positive cells were selected. To isolate single clones, cells were subjected to limited dilution. Individual clones were screened for luciferase and fluorescent protein activities using in vivo imaging. Selected clones were maintained without puromycin for 4 weeks and checked protein activities.

Animals and Tumor Cell Implantation

All the procedures for animal care and tumor cell implantation followed the approved animal protocols and guidelines of the Institutional Animal Care and Use Committee. Prior to implantation, all tumor cells were tested for the presence of mycoplasma and mouse pathogens. Female nude mice (Charles River, Wilmington, MA) were anesthetized with isoflurane and were injected with 3 x 106 cells into mammary fat pads of female nude mice. Tumor growth was monitored using bioluminescence (BLI) and fluorescent (FL) images. Images were taken using an IVIS Spectrum at post-implantation day 15.

Results

Figure 2. Animals and Tumor Cell Implantation

- All the procedures for animal care and tumor cell implantation followed the approved animal protocols and guidelines.
- Tumor cell lines were implanted into mammary fat pads of female nude mice. Tumor growth was monitored using bioluminescence (BLI) and fluorescent (FL) images. Images were taken using an IVIS Spectrum at post-implantation day 15.

Figure 3. Reconstruction of Fluorescent Images Dually Labeled 4T1 Cells

- Dual labeled 4T1-luc2-tdTomato cells were generated. These cells are used to monitor tumor growth by bioluminescence and fluorescence imaging.
- In vivo bioluminescent and fluorescent imaging showing the localization of dual labeled 4T1-luc2-tdTomato cells.

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