In Vivo Monitoring of Fluorescently Labeled Cancer Cells

Eva Barton, Angel Ang, Konnie Urban, Chainy Kuo, Kevin Francis, and Jae-Beom Kim
Caliper Life Sciences, Alameda, CA, USA

Abstract
Whole animal in vivo imaging has contributed significantly to the detection of disease progression and drug efficacy for the past several years. With the introduction of various imaging modalities like bioluminescence and fluorescence, the emphasis has been to develop the most sensitive imaging modality in vivo. With the development of novel imaging techniques like bioluminescence and fluorescence, we have developed novel imaging techniques that can detect single intact cells in vivo. To expand the application of genetically labeled fluorescent tumor cells, we have used a combination of bioluminescence and fluorescence to detect tumor growth in vivo. The authors thank KD Modi and Drs. Mark Roskey, Rajendra Singh, and Brad Rice for their helpful discussions.

Results

Cell Culture and Growth Curve Generation
Human breast cancer cell line MDA-MB-231 Cells were grown in high glucose RPMI 1640 medium (ATCC) and Eagle’s Minimum Essential Medium (ATCC) supplemented with 10% fetal bovine serum (Invitrogen, L5030). To isolate single cell colonies, cells were suspended in 0.25% trypsin and counted using an automatic cell counter (Thermo, Waltham, MA). Total number of cells were plated in a 24-well plate for each implantation. A representative mouse images are shown. Fluorescent and bioluminescent images were processed using spectral analysis software. Fluorescent and bioluminescent images were taken immediately after adding the substrate into the cells using a cooled CCD camera. Light outputs were quantified using Living Image® software. Fluorescent images were taken using a cooled CCD camera. Light outputs were quantified using Living Image® software.

References

Acknowledgments
The authors thank KD Modi and Drs. Mark Roskey, Ramesh Singh, and Brad Rice for their helpful discussions.

Material and Methods

Generation of Fluorescent Protein Expression Vector
Enhanced luciferase I (luc2) OM4 was from plasmid 1.1 vector (Promega, MI). Luc2 plasmids were electroporated into pcDNA3.1 (Invitrogen, Carlsbad, CA) and transfected into parental luc2 cell lines (4T1-luc2, PC3M-luc2, and MDA-MB-231-luc2). Cells were transfected with the tdTomato vector to develop dual labeled cell lines (4T1-tdTomato, PC3M-tdTomato, and MDA-MB-231-tdTomato). Cells were monitored for metastases. 29 days post-implantation, fluorescent images were taken using a cooled CCD camera. Light outputs were quantified using Living Image® software. Bioluminescent images were taken immediately after adding the substrate into the cells using a cooled CCD camera. Light outputs were quantified using Living Image® software.

Summary

1. We have generated single- and dual-labeled tumor cell lines.
2. Both bioluminescence and fluorescence can be used to measure tumor growth and metastasis in vivo.
3. Fluorescently labeled protein probes can be targeted tumors in vivo and can be imaged live without in vivo imaging.
4. Fluorescent protein and luciferase can be unlabeled in three dimensionally reconstituted fluorescent images.