Quantitative Tomographic In Vivo Imaging of Syngeneic Breast Cancer Metastasis to the Lung and Therapeutic Response

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1 Abstract

Breast cancer is a clinical challenge today, with almost 200,000 new breast cancer cases reported annually in the United States alone. In 10% of breast cancer diagnoses, the cancer has metastasized to distant organs in the body such as bone, liver, and lung, decreasing the 5-year relative survival rate to 20%. It is therefore essential to develop robust in vivo imaging approaches that can help dissect the metastatic process and assist in the development of effective targeted therapeutic agents. Using imaging and agent approaches that are translatable from preclinical to clinical application further strengthens the utility of such efforts.

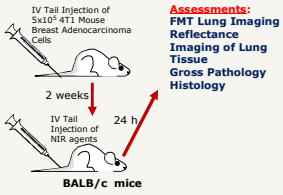
To best establish robust imaging measures of the metastatic disease process in a model resembling clinical disease, we used 4T1 mouse breast adenocarcinoma cells injected intravenously into normal, immunologically-competent BALB/c mice. Three near infrared imaging agents, ProSense® 750 (PerkinElmer), a cathepsin-activatable agent, IntegriSense® 680 (PerkinElmer), an integrin-targeted agent and AngioSense® 680 (PerkinElmer), a vascular agent, were injected IV to detect the protease activity and vascular leak associated with aggressive breast cancer growth. Disease-specific fluorescence was imaged and quantified in living animals using the optical Fluorescence Molecular Tomography – or FMT™ (FMT 2500™ quantitative tomography system, PerkinElmer), showing a consistent and significant increase in ProSense signal as early as 7-10 days, with accompanying increases in lung weight, a current standard measure in lung metastasis models. A standard clinical treatment using 5-Fluorouracil/2'-deoxyinosine (5-FU/2DI) significantly reduced ProSense-related fluorescence in the lung with greater sensitivity than seen with decreases in lung weight. In addition, treatment with a small molecule integrin antagonist decreased both AngioSense and ProSense signal more effectively than seen by changes in lung weight.

Quantification of protease activity and vascular leak provided important non-invasive measures of relevant tumor biology for comparison to standard measures of lung weight and histology and also illustrated the efficacy of clinical therapy. These data clearly demonstrate that deep tissue metastatic growth and response to treatment can be monitored *in vivo* in real time with a near infrared imaging agents and FMT quantitative tomography. The ability to use fluorescent imaging agents operating in the near infrared range also allow the possibility for clinical translation of these techniques.

2 Results

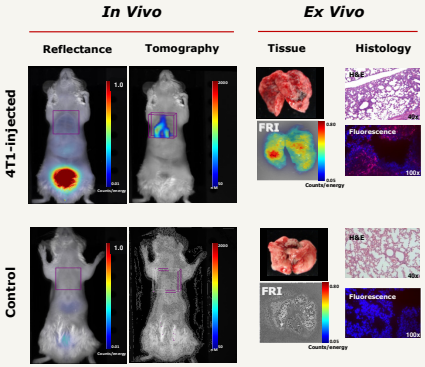
Figure 1: Experimental protocol

4T1 Breast Cancer Lung Metastasis Model



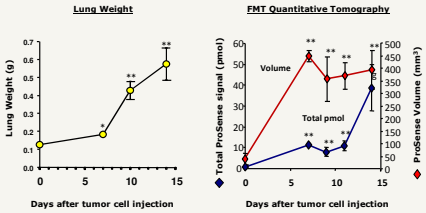
BALB/c mice were injected intravenously with 0.5 - 5x10^5 4T1 mouse breast tumor cells, and mice were injected with either AngioSense® 680, ProSense® 750, or Integrisense® 680 to detect tumor perfusion/blood pooling, cathepsin activity, or $\alpha_v\beta_3$ integrin expression, respectively. Twenty four hours later, mice were imaged tomographically using FMT imaging to detect and quantitate levels of activated agent within the tumor tissue. Mice were then sacrificed, lungs collected, and macroscopic images collected using digital photography and Fluorescent Reflectance Imaging (FRI). Finally, lungs were fixed, sectioned, and stained with H&E for histological analysis.

Figure 2: FMT 2500 Quantitative Fluorescence Imaging of Tumor Cathepsin Activity



Two weeks following injection of 5x10^5 4T1 cells, mice were injected with ProSense® 750 and imaged by FMT 24 hrs later. Three dimensional regions of interest (ROIs) were drawn to encompass the lung areas using the FMT imaging system software. Fluorescent signal attributable to ProSense metabolism (Liver, GI tract, bladder) is excluded from the tomographic images. Metastatic tumor growth in the lungs of 4T1-injected is apparent by qualitative fluorescence reflectance imaging (FRI), H&E, and fluorescence microscopy of lung tissue.

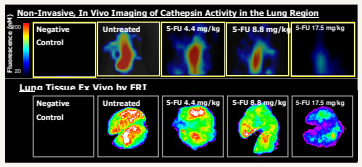
Figure 3: Quantification of Tumor Cathepsin Activity



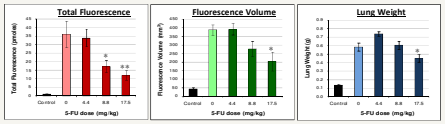
At different times after inoculation with 4T1 cell, mice were injected with ProSense® 750 and imaged by FMT 24 hrs later. Analysis of tomographic lung imaging datasets was performed using careful thresholding based on minimizing background signal in the negative control animals (due to low-level cathepsin activity in normal resident macrophages). ProSense fluorescence volume and total picomoles within the lungs of 4T1 recipients were quantified using the FMT 2500 system software.

Figure 4: Quantification of 5-FU Efficacy with ProSense® 750

Whole Animal FMT and Tissue Reflectance Imaging

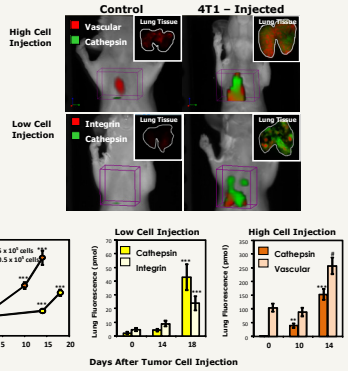


FMT Quantification



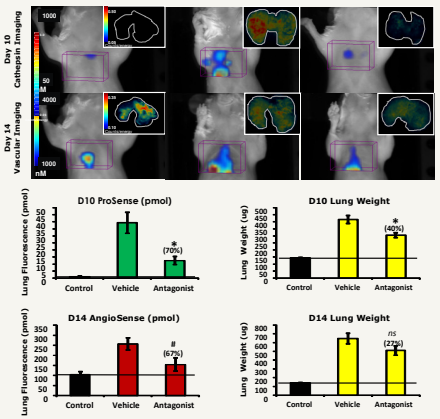
Mice received 5-Fluorouracil (5-FU) + Deoxyinosine (dino), at doses and dosing regimens indicated, beginning one day following 4T1 cell injection. On day 13, mice received a dose of ProSense® 750 and were imaged by FMT 24h later. 5FU+dino significantly reduced metastatic tumor as assessed by lung weight, total lung fluorescence (FMT), lung fluorescence volume (FMT), and tissue FRI, with total fluorescence showing the greatest sensitivity.

Figure 5: Multiplex FMT 2500 Quantification of Vascular Leak, Cathepsin Activity, and Integrin Expression



At different time points after inoculation with either 0.5 x 10^5 or 5 x 10^5 4T1 cells, mice were injected with ProSense®750 (Cathepsin activity), IntegriSense®680 ($\alpha_v\beta_3$ integrin expression), or AngioSense®680 (vascular leak) and imaged by FMT 2500 24h later. Two-color representation of FMT 2500 tomography and FMT 2500 FRI imaging shows regions of biological co-localization.

Figure 6: Multiplex FMT 2500 Quantification of Integrin Antagonist Efficacy



Mice received an i.p injection of a small molecule integrin antagonist (25 mpk/mouse/b.i.d.) starting at day 0, starting 1h prior to 4T1 cell injection. On days 9 and 13, mice received a combined dose of AngioSense® 680 and ProSense® 750 and were imaged by FMT 24h later. The antagonist significantly reduced metastatic tumor as assessed by lung weight, total lung fluorescence (FMT 2500), lung fluorescence volume (FMT), and tissue FRI (FMT 2500 FRI). ProSense showed the greatest therapeutic effect with Day 10 imaging, whereas AngioSense detected no therapeutic benefit until Day 14.

3 Summary

Mouse models of cancer metastasis rely predominantly on ex vivo tissue weight, nodule counts, and/or histologic analysis for the assessment of tumor burden. To assess the feasibility of non-invasive, quantitative imaging of deep tissue metastases, we used different NIR agents to image vascular leak, upregulation of cathepsins, and increases in integrin expression, known to occur in cancer progression and metastasis. We found that 4T1 cell injection led to diffuse, wide-spread metastases within the lungs of recipient mice, rather than distinct small metastases. Severity of the disease progression was modified using different 4T1 cell doses. Fluorescent tomographic imaging (FMT) allowed the quantification of fluorescent agents accumulating in the tumors, with this quantification yielding better results than typical lung weight assessment. Treatment efficacy with either 5-FU or a small molecule integrin antagonist led to robust and quantitative changes, with the additional capacity of measuring multiple biological changes simultaneously. These findings were confirmed by reflectance imaging of *ex vivo* tissue and by histology. With this model we have established the potential utility of quantitative fluorescence tomography (FMT) in preclinical drug discovery in the challenging area of deep tissue metastasis.

4 References

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Grimm J, Kirsch DG, Windsor SD, Kim CF, Santiago PM, Ntziachristos V, Jacks T, Weissleder R. Use of gene expression profiling to direct in vivo molecular imaging of lung cancer. *Proc Natl Acad Sci U S A.* 2005;102:14404-14409.

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