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PET Imaging of a Marker for Breast Cancer Metastasis

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PET Imaging of a Marker for Breast Cancer Metastasis

The serine protease matriptase has been implicated in epithelial cancers, and has been indicated as a biomarker for survival independent of HER-2/neu. While in vitro methods are invaluable, few breast cancer cell lines express matriptase, though a majority of breast cancers are positive for matriptase—this suggests that imaging the in vivo behavior of matriptase may aid in understanding its context in a tumor system. Specifically, activated matriptase is associated with cancer progression. We have developed tracers against activated matriptase for in vivo imaging using PET. M69 antibody (against activated matriptase) was functionalized to capture $^{64}$Cu: $^{64}$Cu-TETA-M69 was evaluated in mouse models of human breast cancer. In a tet-regulable model for human matriptase expression, with confirmation from IHC, we found marker specific tumor retention through biodistribution and PET imaging. In summary, we have developed a novel radiotracer for the first imaging of activated matriptase in vivo, demonstrating favorable pharmacokinetics. This approach has the potential for imaging metastasis, the primary cause of mortality in breast cancer patients.

human breast cancer, matriptase, serine protease, positron emission tomography (PET), immunoPET
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Introduction

Matriptase is a type II transmembrane serine protease is primarily found in the epithelium, the origin of the majority of adult cancers, and is an oncogenic enzyme (List et al., 2005; Oberst et al., 2003). Matriptase has been found overexpressed in several cancers, including breast cancer, and it is hypothesized that the dysregulation of this enzyme occurs with an imbalance with the cognate inhibitor, hepatocyte growth factor activator inhibitor 1 (HAI-1) (Benaud et al., 2002). After matriptase is transformed from the latent (single-chain) to the activated (two-chain) state, it forms a reversible complex with HAI-1. This activated state is implicated with cancer progression and metastasis, where high expression of Met (the receptor for HGF), matriptase and HAI-1 was associated with poor patient outcome (Kang et al., 2003). Matriptase also has optimal activation under acidic conditions (pH 5.8 - 6.0). Acidic tissue is also observed under the increased levels of aerobic glycolysis in cancerous tissue, and this phenomenon is responsible for the contrast observed with tumor uptake of the most commonly used positron emission tomographic (PET) radiotracer used in clinical oncology. \(^{18}\)F-fluorodeoxyglucose (FDG) (Gatenby & Gillies, 2004; Lee et al., 2007).

Matriptase is found in a majority of frozen tissues from breast cancers, but this is not reflected in the cell-lines that model this disease (Bhatt et al., 2003). This suggests that the molecular in vivo imaging with PET of this novel cancer target may be a valuable approach for further understanding this protein in a manner that may currently not be available via in vitro methods. Imaging activated matriptase may allow for a means to measure the extent of breast cancer metastasis, the primary cause of mortality in patients with the disease. The primary goal of this predoctoral work was the development and evaluation of a PET-based radioimmunoconjugate specific against activated matriptase.
Our specific aims were:

1. To develop a monoclonal antibody (mAb)-based imaging agent (radioimmunoconjugate) to target activated matriptase.

2. To perform \textit{in vivo} small animal PET imaging of activated matriptase.

3. To evaluate \textit{ex vivo} whether regions of imaging contrast correlate with target expression.

\textbf{Body}

The monoclonal antibody (mAb) M69 specifically recognizes activated matriptase (Benaud \textit{et al.}, 2001), and was conjugated to a chelator that has specificity the capture of copper, 1,4,8,11-tetraazacyclotetradecane-N,N',N''-tetraacetic acid (TETA) (McCall \textit{et al.}, 1990). Copper-64 (Cu-64) is a metal that also a positron emitting radioisotope suited for PET imaging. A representation of the chelate conjugated to a mAb (\textsuperscript{64}Cu-TETA-M69) is shown in panel (a) of Figure 1. Using immunoblots with the labeled radioimmunoconjugate \textsuperscript{64}Cu-TETA-M69, and chelate-to-antibody ratio assays through thin-layer chromatography (radio-TLC), conditions were optimized under which, respectively, the immunoreactivity of radioimmunoconjugates was retained, and properties of lower predicted liver uptake were obtained (panel (b) of Figure 1).

During this year in funding, a polyoma middle T model cell-line that has a tetracycline (dox)-regulable expression of human matriptase was inoculated subcutaneously in female nude mice. This formed a tumor that has the combined properties of a breast cancer model with a predictable metastasis to lung and the regulable expression of the human biomarker. The correlated expression of matriptase controlled by the presence of dox in the growth media is demonstrated in Figure 2. Both panels
Figure 1. (a) Diagram highlighting TETA chelate structure and covalent bond to mAb and (b) evaluation of immunoreactivity by immunoblotting of matriptase with $^{64}$Cu-TETA-M69, a Cu-64 labeled radioimmunoconjugate specific against activated matriptase, at concentrations ranging from 5 to 50 $\mu$/ml. Dark bands provide evidence that this radioimmunoconjugate retained immunoreactivity despite the conjugation chemistry.

show immunofluorescence staining for matriptase (green), combined with nuclear counterstain (blue), when cells are grown in the presence (left) and absence (right) of dox (Figure 2).

For biodistribution studies, tumored mice were split into two cohorts, where one group was standard chow, and the other group fed dox-containing feed, and were sacrificed for necropsy with tissues and organs measured for activity with a $\gamma$-counter and normalized by tissue weight (percent injected dose per gram, or % ID/g). For imaging studies, mice were fed standard chow one week and imaged at 24h intervals through four days with small-animal PET, after the intravenous administration of $^{64}$Cu-TETA-M69 formulated in saline (left panel of Figure 3). Mice were then changed onto the dox-containing feed, re-administered $^{64}$Cu-TETA-
Figure 2. Confocal immunofluorescence of tet-regulable stained for matriptase expression, when grown in the presence (left) and absence (right) of the tetracycline doxycycline (dox) shown in green (Alexa Fluor 488) with blue nuclear counterstain (DAPI).

M69, and imaged again with small-animal PET (same mouse is shown in right panel of Figure 3). Both of these panels were taken at three-day timepoints after the introduction of the radioimmunoconjugate, and co-registered with a computed tomography (CT) scan for anatomical orientation.

While these PET-CT fused volume images reveal some liver uptake, the tumor shows a significant increase in uptake or retention of the radioconjugate that correlated with the change to dox feed (as indicated by the arrows in Figure 3). The panels of Figure 4 show a comparison of the normalized tumor uptake from standard (yellow) and dox chow (green) fed mice, where panel (a) is a time-series of boxplots with values from the biodistribution study, and panel (b) is a series of boxplots with tumor uptake extracted from volumes-of-interest in PET volumes. Both biodistribution and imaging show a similar trend of increased tumor uptake associated with the change to dox feed. With small-animal PET imaging, a considerably smaller cohort of animals was required in comparison with biodistribution studies, and the same mouse could be followed non-invasively through more time points under different feed conditions, and this highlights a significant advantage of PET. Several animals exhibited lung metastases that were found by necropsy, and the possibility of PET in the detection of these metastases is currently under evaluation.
**Figure 3.** Imaging shown for $^{64}$Cu-TETA-M69 in a representative nude female mice with the Tet regulable polyoma middle T matriptase (20FLM) single tumor model. The same mouse was first imaged on standard feed, and changed onto dox containing feed the week after. There appeared to be a corresponding increase in tumor uptake/retention associated with the change in feed.

Immunohistochemical staining of matriptase is the gold standard to verify protein expression, and was used to follow up on the increased tumor accumulation of contrast agent. The left column of images are hematoxylin and eosin stains of tumor sections. These are shown in comparison with the following three columns, which are representative examples of immunostaining for matriptase, given in order of increasing magnification (Figure 5). In tumor sections taken from mice administered dox feed (example shown in top row), there was increased matriptase expression, demonstrated through a characteristic membrane staining, shown in brown (Figure 5). The bottom row is from a representative control mouse on standard feed, and no such pattern for staining was observed (Figure 5). This immunohistochemistry confirms that the increased PET signal observed in the tumors of dox-fed mice indeed is correlated with an increase in matriptase expression.
Figure 4. Boxplot summary of tumor uptake in single tumor model of $^{64}\text{Cu}$-TETA-M69 measured from (a) biodistribution and (b) imaging.
Figure 5. Representative immunohistochemical stains of 20FLM tumor. Hematoxylin and eosin (H & E) and total matriptase stained tumor FFPE sections derived from 20FLM tumors in the presence (a) through (d); and absence (e) through (g) of doxycycline feed. Note that the top row represent tissue sections taken from the identical mouse shown in Figure 3 after the transition to dox-containing feed. The bottom row is a representative control mouse that received standard feed alone.
**Key Research Accomplishments**

1. Synthesis, radiolabeling and evaluation of radioimmunoconjugates against activated matriptase

2. Small animal PET imaging of these copper-64 labeled radioimmunoconjugates administered intravenously in a mouse model for human breast cancer

3. Correlation by biodistribution and immunohistochemistry to confirm that regions of increased image contrast observed with PET indeed correlated with elevated matriptase expression

**Reportable Outcomes**

**Training Accomplishments**

1. Attendance at Mouse Club meetings with forum for the intersection of breast cancer researchers and discussion, including pathologists, biologists, and engineers.


**Journal Papers (in progress)**


bifunctional chelators used in the imaging of a novel cancer molecular target. Manuscript in preparation. Content: Comparison of the chelators DOTA and TETA.


Conference Presentations and Abstracts


**Honors and Awards**

1. 56th Annual Meeting of the Society of Nuclear Medicine (SNM), Toronto, Canada. Radiopharmaceutical Sciences Council (RPSC) Young Investigator Award Symposium (first prize) (travel award and honorarium, 2009).

2. Graduate Student Association (UC Davis) Travel Award (support for SNM conference, 2009).

3. Biomedical Engineering Society (BMES) Travel Award (support for BMES conference, 2009).

4. Graduate Studies (UC Davis) Travel Award (support for AACR Mol Targets conference, 2009).
Conclusion

During the period from 1 January 2009 through 31 January 2010, I have had the combined experiences of my first opportunities to attend cancer research associated meetings, including the Society of Nuclear Medicine and the Molecular Targets and Therapeutics meeting of the American Association for Cancer Research (AACR). It is also with the support of this program that has provided the opportunity to follow through the idea of the molecular targeting of activated matriptase from conception through the preclinical stage in a mouse model for human breast cancer as my graduate work.

In summary, we have developed a radioimmunoconjugate against activated matriptase, and have demonstrated that activated matriptase may be feasible as a PET imaging target, and this suggests that activated matriptase may be a valid tumor biomarker that can be further explored for clinical relevance. A source of motivation for future development is the potential for use with triple-negative breast cancer patients, who being estrogen, progesterone and HER-2 negative, currently do not have markers that can be used for diagnosis or to detect early responses to treatment.
References


Exposure in C57BL/6 mice. *International Journal of Radiation Oncology*Biology*Physics, 69, 1272–1281. (document)


Appendix: Conference Abstracts in 2009

Abstract 1: 56th Annual Meeting of the Society of Nuclear Medicine (oral)

Title: PET imaging of activated matriptase, a marker for cancer progression.
Authors: J. Choi¹, S. Hausner¹, M. Gagnon¹, D. Kukis², J. Holland³, J. Lewis³, C. Lin⁴, M. Johnson⁵, J. Sutcliffe¹,². 1. UC Davis, Dept of Biomedical Engineering, Davis, CA 2. UC Davis, Ctr for Molecular and Genomic Imaging, Davis, CA 3. Memorial Sloan-Kettering Cancer Ctr, New York, NY 4. Univ of Maryland, School of Medicine, Baltimore, MD 5. Georgetown Univ, Dept of Oncology, Lombardi Comprehensive Cancer Ctr, Washington, DC

Objectives: Activated matriptase is a serine protease associated with cancer progression. We propose to develop radioimmunoconjugates against activated matriptase for in vivo imaging using PET. Methods: M69 antibody (against activated matriptase) was functionalized to capture [⁶⁴Cu]copper or [⁸⁹Zr]zirconium: ⁶⁴Cu-TETA-M69 and ⁸⁹Zr-Df-M69 were evaluated in vivo. Briefly, a tet-regulable cell-line (human breast cancer model- derived) was introduced into female nude mice, and fed dox chow (+dox) for matriptase- positive or normal chow (-dox) for control tumors. Mice were imaged with microPET at 24, 48, 72, and 96h; and through 120, 144, 192, and 288h for ⁸⁹Zr; corresponding biodistribution studies were performed.

Results: Radioimmunoconjugates were >95% radiochemical purity and immunoreactive. PET images and biodistribution indicated specific tumor retention with the tet-regulable model; however, more liver uptake for ⁸⁹Zr-Df-M69 was observed than for ⁶⁴Cu-TETA-M69. Biodistribution at 96h revealed ⁶⁴Cu-TETA-M69 tumor-to-blood ratios of 2.25 and 2.01; and 2.91 and 1.47 for ⁸⁹Zr-Df-M69, for +dox and -dox, respectively. At 310h, ratios were 9.14 and 5.16 for ⁸⁹Zr-Df-M69. Conclusions: We have developed two radioimmunoconjugates for imaging activated matriptase.
in vivo. This approach has the potential for imaging metastasis, the primary cause of mortality in breast cancer patients. Research Support: JCC is supported by the Department of Defense Breast Cancer Research Program under award number W81XWH-08-BCRP.

Abstract 2: 56th Annual Meeting of the Society of Nuclear Medicine (poster)

Title: Imaging regulable expression of matriptase in a mouse model for human breast cancer with PET.

Authors: J. Choi¹, S. Hausner¹, M. Gagnon¹, D. Kukis², C. Lin³, M. Johnson⁴, J. Sutcliffe¹,².

1. UC Davis, Dept of Biomedical Engineering, Davis, CA 2. UC Davis, Ctr for Molecular and Genomic Imaging, Davis, CA 3. Univ of Maryland, School of Medicine, Baltimore, MD 4. Georgetown Univ, Dept of Oncology, Lombardi Comprehensive Cancer Ctr, Washington, DC

Objectives: The serine protease matriptase has been implicated in many epithelial cancers. We propose to develop radioimmunoconjugates to image in vivo expression of both the activated and total states of matriptase. Methods: The radioimmunoconjugates ⁶⁴Cu-TETA- M69 (activated) and ⁶⁴Cu-TETA-M32 (total matriptase) were synthesized and evaluated in vivo. Briefly, female nude mice were implanted with a tetracycline (dox)-regulable cell line (3E6 cells), and fed dox chow for matriptase-positive expressing tumors or standard chow for control tumors. Mice were injected with ⁶⁴Cu-TETA-M69 or ⁶⁴Cu-TETA-M32 (50-150 μCi, 20 μg) and imaged using microPET at 24, 48, 72 and 96h; corresponding biodistribution studies were also performed. Results: ⁶⁴Cu-labeled immunoconjugates were >95% radiochemically pure and immunoreactive. PET images showed specific accumulation of both immuno-
conjugates in target positive tumors. Biodistribution revealed a two-fold increase in tumor activity from dox-fed mice over those fed normal chow for $^{64}$Cu-TETA-M32 at 96h, with a more modest uptake for $^{64}$Cu-TETA-M69. **Conclusions:** We have demonstrated that tet-regulable matriptase expression can be monitored in vivo using PET. While faster tumor targeting was observed for the total state, activated matriptase is the more relevant target for future clinical development. We observed an upward trend of specific tumor uptake at 96h; longer-lived isotopes may be required for improved detection of activated matriptase. **Research Support:** Julia C. Choi is supported by the Department of Defense Breast Cancer Research Program under award number W81XWH-08-BCRP.

**Abstract 3: UC-wide Bioengineering Symposium (oral)**

**Title:** Imaging of regulable expression of matriptase, a marker for cancer progression in a mouse model for human breast cancer with PET

**Authors:** J. C. Choi$^1$, S. H. Hausner$^1$, M. K. J. Gagnon$^1$, D. L. Kukis$^2$, C.-Y. Lin$^3$, M. D. Johnson$^4$, J. L. Sutcliffe$^{1,2}$.

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The serine protease matriptase has been implicated in many epithelial cancers. We propose to develop radioimmunoconjugates to image in vivo expression of both the activated and total states of matriptase using microPET. The radioimmunoconjugates $^{64}$Cu-TETA-M69 (against activated matriptase) and $^{64}$Cu-TETA-M32 (against total matriptase) were synthesized and evaluated in vivo. Briefly, female nude mice were implanted with a tetracycline (dox)- regulable cell line (3E6 cells), and fed dox chow
for matriptase-positive expressing tumors or standard chow for control tumors. Mice were injected with $^{64}$Cu-TETA-M69 or $^{64}$Cu-TETA-M32 (50-150 $\mu$Ci, 20 $\mu$g) and imaged using microPET at 24, 48, 72 and 96h; corresponding biodistribution studies were also performed. Biodistribution and image values were evaluated for statistical significance with general linear model testing. $^{64}$Cu-labeled radioimmunoconjugates were > 95% radiochemically pure and immunoreactive. PET images showed specific accumulation of both immunoconjugates in target positive tumors. Biodistribution revealed a two-fold increase in tumor activity from dox-fed mice over those fed normal chow for $^{64}$Cu-TETA-M32 at 96 h, with a more modest uptake for $^{64}$Cu-TETA-M69. We have developed two radioimmunoconjugates for imaging activated matriptase in vivo. We have demonstrated that tet-regulable matriptase expression can be monitored in vivo using PET. While faster tumor targeting was observed for the total state, activated matriptase is the more relevant target for future clinical development. We observed an upward trend of specific tumor uptake at 96h; longer-lived isotopes may be required for improved detection of activated matriptase. This approach has the potential for imaging metastasis, the primary cause of mortality in breast cancer patients. JC is supported by the Department of Defense Breast Cancer Research Program under award number W81XWH-09-1-0013.

Abstract 4: Biomedical Engineering Society (BMES) Annual Fall Meeting (oral)

**Title:** Quantitative imaging of regulable expression of matriptase in a model for human breast cancer with PET

**Authors:** J. C. Choi$^1$, S. H. Hausner$^1$, M. J. Gagnon$^1$, D. L. Kukis$^1$, J. P. Holland$^2$, J. S. Lewis$^2$, C.-Y. Lin$^3$, M. D. Johnson$^4$, J. L. Sutcliffe$^1$.

$^1$University of California, Davis, Davis, CA, $^2$Memorial Sloan-Kettering Cancer Center,
**Introduction:** The serine protease matriptase has been implicated in epithelial cancers. We have developed tracers to image *in vivo* expression of both the activated and total states of matriptase using microPET.

**Methods:** The radioimmunoconjugates $^{64}$Cu-TETA-M69 and $^{89}$Zr-DF-M69 (activated matriptase) and $^{64}$Cu-TETA-M32 (total) were synthesized and evaluated *in vivo*. Briefly, female mice were implanted with a tetracycline (dox)-regulable cell line, and fed dox chow for matriptase-positive expressing tumors or standard chow for control tumors. Mice were injected with $^{64}$Cu-TETA-M69, $^{89}$Zr-DF-M69 or $^{64}$Cu-TETA-M32 and imaged (1-12 days); corresponding biodistribution studies were performed. Data were evaluated for statistical significance with general linear model testing.

**Results:** $^{64}$Cu- and $^{89}$Zr-labeled tracers were >95% radiochemically pure and immunoreactive. PET images showed specific accumulation of agents in target positive tumors; quantitation revealed a 40% increase in tumor activity from dox-fed mice over those fed normal chow for $^{64}$Cu-TETA-M69 and $^{89}$Zr-DF-M69, with a comparable differential observed for $^{64}$Cu-TETA-M32.

**Conclusions:** We have developed tracers for imaging matriptase *in vivo*, and have demonstrated that tet-regulable matriptase expression can be monitored using PET. While faster tumor targeting was observed for the total state, activated matriptase is the more relevant target for clinical development. This holds the potential for imaging metastasis, the primary cause of mortality in breast cancer patients. JC is supported by the Department of Defense Breast Cancer Research Program under award number W81XWH-09-1-0013.
Abstract 5: AACR-EORTC-NCI Molecular Targets and Cancer Therapeutics (poster)

Title: Positron emission tomographic (PET) imaging of activated matriptase, a marker for cancer progression

Authors: Julia C. Choi, Sven H. Hausner, M. Karen J. Gagnon, David L. Kukis, Jason P. Holland, Jason S. Lewis, Chen-Yong Lin, Michael D. Johnson, Julie L. Sutcliffe.
University of California, Davis, Davis, CA, Memorial Sloan-Kettering Cancer Center, New York, NY, University of Maryland, Baltimore, Baltimore, MD, Georgetown University, Washington, DC

Abstract: The serine protease matriptase has been implicated in epithelial cancers, has been found in ~45% of node-negative breast cancers at high levels, and is indicated as a biomarker for survival independent of HER-2/neu. While in vitro methods are invaluable, few breast cancer cell-lines express matriptase, though 83% of breast cancer patients are positive for matriptase—this suggests that imaging the in vivo behavior of matriptase may aid in understanding its role in the context of the tumor system. Specifically, activated matriptase is associated with cancer progression. We have developed radiotracers against activated matriptase for in vivo imaging using PET. M69, an antibody against activated matriptase, was functionalized to capture $^{64}$Cu]copper or $^{89}$Zr]zirconium: $^{64}$Cu-TETA-M69 and $^{89}$Zr-DF-M69 were evaluated in a mouse model of human breast cancer. A dox-regulable pair of PyVmT cell-lines was bilaterally introduced into nude female mice to generate matriptase-positive and control tumors. Mice were fed dox chow ad libitum, administered radiotracer, imaged using microPET at 1-4d p.i.; and through 336h for $^{89}$Zr; corresponding biodistribution was performed. PET images indicated specific tumor retention. Biodistribution at 4d revealed both $^{64}$Cu-TETA-M69 and $^{89}$Zr-DF-M69 retained two-fold uptake ratios for the positive tumor over control; this was also ob-
served at 336h for $^{89}$Zr-DF-M69. Immunohistochemistry on FFPE tissues confirmed human matriptase expression in the positive tumor. In summary, we have developed two novel radiotracers and performed the first *in vivo* imaging of activated matriptase. This approach has the potential for imaging metastasis, the primary cause of mortality in breast cancer patients. JC is supported by the Department of Defense Breast Cancer Research Program under award number W81XWH-09-1-0013.