Published in final edited form as:

Curr Mol Med. 2012 May ; 12(4): 458–466.

Preclinical Molecular Imaging of the Translocator Protein (TSPO) in a Metastases Model Based on Breast Cancer Xenografts Propagated in the Murine Brain

Shelby K. Wyatt^{#,1,2}, H. Charles Manning^{1,2,3,4,5,6}, Mingfeng Bai⁹, Moneeb Ehtesham⁴, Khubaib Y. Mapara⁴, Reid C. Thompson⁴, and Darryl J. Bornhop^{*,6,7,8}

¹Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Nashville, TN, USA

²Department of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN, USA

³Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA

⁴Department of Neurological Surgery, Vanderbilt University Medical Center, Nashville, TN, USA

⁵Chemical and Physical Biology Program, Vanderbilt University, Nashville, TN, USA

⁶The Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA

⁷Department of Chemistry, Vanderbilt University, Nashville, TN, USA

⁸The Vanderbilt Institute for Chemical Biology, Vanderbilt University, Nashville, TN, USA

⁹Department of Radiology and University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Previous studies have demonstrated the feasibility of translocator protein (TSPO) imaging to visualize and quantify human breast adenocarcinoma (MDA-MB-231) cells *in vivo* using a TSPO-targeted near-infrared (NIR) probe (NIR-conPK11195). This study aimed to extend the use of the TSPO-targeted probe to a more biologically relevant and clinically important tumor microenvironment as well as to assess our ability to longitudinally detect the presence and progression of breast cancer cells in the brain. The *in vivo* biodistribution and accumulation of NIR-conPK11195 and free (unconjugated) NIR dye were quantitatively evaluated in intracranial MDA-MB-231-bearing mice and non-tumor-bearing control mice longitudinally once a week from two to five weeks post-inoculation. The *in vivo* time-activity curves illustrate distinct clearance profiles for NIR-conPK11195 and free NIR dye, resulting in preferential accumulation of the TSPO-targeted probe in the intracranial tumor bearing hemisphere (TBH) with significant tumor contrast over normal muscle tissue ($p < 0.005$ at five weeks; $p < 0.01$ at four weeks). In addition, the TSPO-labeled TBHs demonstrated significant contrast over the TBHs of mice injected with free

* Address correspondence to this author at the Department of Chemistry, Vanderbilt University, VU Station B 351822, 7300 Stevenson Center, Nashville, TN 37235-1822, USA; Tel: 615-322-4226; Fax: 615-343-1234; darryl.bornhop@vanderbilt.edu.

[#]Current Affiliation: Department of Biomedical Imaging, Genentech Inc., South San Francisco, CA 94080, USA.

NIR dye ($p < 0.001$ at four and five weeks) as well as over the TSPO-labeled non-tumor-bearing hemispheres (NTBHs) of control mice ($p < 0.005$ at four and five weeks). Overall, TSPO-targeted molecular imaging appears useful for visualizing and quantifying breast cancer xenografts propagated in the murine brain and may assist in preclinical detection, diagnosis and monitoring of metastatic disease as well as drug discovery. Furthermore, these results indicate it should be possible to perform TSPO-imaging of breast cancer cells in the brain using radiolabeled TSPO-targeted agents, particularly in light of the fact that [^{11}C]-labeled TSPO probes such as [^{11}C]-PK 11195 have been successfully used to image gliomas in the clinic.

Keywords

Breast cancer; metastasis; molecular imaging; peripheral benzodiazepine receptor; PK 11195; translocator protein

INTRODUCTION

The American Cancer Society estimated that approximately 226,870 women and 2,190 men would be newly diagnosed with invasive breast cancer in 2012, representing the second most common cancer in women [1]. In addition, approximately 39,510 women and 410 men were expected to die from breast cancer in 2012 [1]. If breast cancer is detected in its early stages, the five-year survival rate is 99%, but rapidly declines to 84% if the cancer has spread to either nearby or distant lymph nodes and reaches a dismal 23% when metastases are found in the organs [1]. Thus, metastasis is regarded as a major cause of morbidity and mortality in the breast cancer patient population.

The most common sites of distant breast cancer metastases include bone, lung, liver, and the central nervous system/brain (reviewed in [2–6]). Although breast cancer metastases to the brain are somewhat less common than those of the bone and lung, they represent the second most common cause of central nervous system metastases and have been found in up to 30–40% of cancer patients at the time of autopsy [26]. In addition, breast cancer patients often have an increased risk of relapse, typically in the form of distant organ metastases, including those of the brain [5], which may present as late as 10–20 years following the initial diagnosis [5]. Furthermore, the neurological implications and lack of effective treatment augment the clinical relevance of breast cancer metastases to the brain.

Although patients with brain metastases often present with clinical symptoms including chronic headache, nausea, and cognitive alterations, diagnosis relies heavily on neuroimaging [2, 4, 7]. Currently, the two most common diagnostic techniques in the clinic involve gadolinium-enhanced magnetic resonance imaging (MRI) and contrast-enhanced computed tomography (CT). Gadolinium-enhanced MRI is typically preferred over contrast-enhanced CT due to its higher sensitivity and ability to distinguish between single and multiple lesions (reviewed in [3]), but requires breakdown of the blood-brain barrier, which is not specific to a particular disease, and may therefore only be sensitive to late stage metastases. Metabolic imaging using 2- ^{18}F fluoro-2-deoxy-glucose positron emission tomography (^{18}F FDG-PET) has also been explored with varied results and is generally not recommended for metastatic imaging [8]. In general, specificity issues and high background

uptake of [^{18}F]FDG by the metabolically active normal brain as well as inherent tumor size limitations substantially limit the use of [^{18}F]FDG-PET for imaging breast cancer brain metastases [3]. Preclinically, the use of MRI for tracking micron-sized iron oxide particles (MPIO)-labeled and ferumoxides-protamine sulfate (FEPro)-labeled MDA-MB-231BR cells following intracardiac injection has been demonstrated [9–12]. Although this approach assists in the noninvasive study of metastatic progression in animal models, it requires that the cells be extrinsically labeled with MPIO or FEPro prior to administration rather than targeting an intrinsically upregulated receptor or protein and is therefore not clinically translatable.

As an alternative approach to traditional oncologic imaging, our laboratory has focused on the development of molecular imaging agents that target the translocator protein (TSPO), previously termed the peripheral benzodiazepine receptor (PBR) [13–18]. The TSPO represents an attractive target for molecular imaging of disease due to its upregulation in many neurodegenerative diseases and cancers, including breast cancer [19, 20]. Clinically, the expression of TSPO appears to correlate with aggressive phenotype [20] and is a strong predictor of poor prognosis [21]. Recently, we conducted the first study to demonstrate the feasibility of molecular imaging of the TSPO as a potential approach to breast cancer imaging [18]. Here, we extend the use of the TSPO-targeted probe to a more biologically relevant and clinically important tumor microenvironment, namely MDA-MB-231 breast cancer xenografts propagated in the murine brain, and assess our ability to longitudinally detect the presence and progression of breast cancer cells in the brain.

MATERIALS AND METHODS

Materials

The IRDye® 800CW NHS Ester dye and IRDye® 800-acid (free NIR dye) were acquired from LI-COR Biosciences (Lincoln, Nebraska). MDA-MB-231 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Calcium- and magnesium-free phosphate buffered saline (CMF- PBS), Leibovitz's L-15 media supplemented with 2 mM L-glutamine, fetal bovine serum (FBS), and gentamicin sulfate were purchased from Invitrogen Corporation (Carlsbad, CA). Female athymic nude (nu/nu) mice were obtained from Charles Rivers Laboratories, Inc. (Wilmington, MA).

TSPO-Targeted Molecular Imaging Agent Synthesis and Characterization

NIR-conPK11195 was synthesized, purified and characterized as previously described [14, 18]. The absorbance and emission spectra of free (unconjugated) NIR dye and NIR-conPK11195 were measured using a Shimadzu UV-VIS 1700 spectrophotometer (Columbia, MD) and a PTI Technologies spectrofluorometer (Birmingham, NJ), respectively.

Cell Culture

MDA-MB-231 (human metastatic breast adenocarcinoma) cells were propagated in seal plug culture flasks at 37°C and 95% humidity in Leibovitz's L-15 medium supplemented with 2 mM L-glutamine, 10% FBS, and 50 mg gentamicin. The culture medium was

replaced approximately every three days or as necessary and the cells were sub-cultured approximately 1:2 to 1:10 after attaining confluence.

Animal Models

Animal studies were performed under guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at Vanderbilt University.

A mouse model that mimics breast cancer metastasis to the brain was prepared by stereotactically injecting MDA-MB-231 cells (1×10^6 cells in 4 μ L of sterile saline) into the basal ganglia of female athymic nude (nu/nu) mice to a depth of 2 mm. Although this may not be the optimal animal model of metastasis, it represents the first step towards assessing the feasibility of using a NIR-labeled TSPO-targeted probe to monitor breast cancer metastases to the brain. Following injection, the skin was closed with sterile surgical staples, which were removed after seven days following confirmation of healing.

In Vivo Imaging Studies

Tumor-bearing and non-tumor-bearing mice were injected with 5 nmoles of either free NIR dye or NIR-conPK11195 in sterile saline *via* the tail vein (100 μ L) two weeks post-implantation. The biodistribution and accumulation of the free NIR dye and TSPO-targeted probe were monitored in real-time using the IVIS Imaging System 200 (Xenogen Corporation/Caliper LifeSciences; Alameda, CA) equipped with an indocyanine green (ICG) excitation and emission filter set (710 – 760 nm and 810 – 875 nm, respectively). Images were obtained pre-injection and approximately five minutes, 4.5 hours, 8.5 hours, 12.5 hours, and 24.5 hours post-injection using the following instrument settings: 7 seconds exposure time, medium binning (8 $\times$ 8 pixels), f/stop of 8 and FOV D (19.5 cm x 19.5 cm). The mice were then imaged longitudinally once a week for three consecutive weeks. On the fifth week post-implantation, the mice were sacrificed approximately 25 hours post-injection of NIR-conPK11195 or free NIR dye and the harvested brains were imaged using the following IVIS instrument settings; 3 seconds exposure time, medium binning (8 $\times$ 8 pixels), f/stop of 8 and FOV B (6.5 cm x 6.5 cm). The brains were subsequently fixed in 4% paraformaldehyde overnight, followed by ethanol dehydration. The fixed tissues were then paraffin embedded, sliced and stained with hematoxylin and eosin (H&E) for histological characterization.

During the imaging sessions, the mice were kept on a heated animal support plate under general anesthesia by inhalation of 2–3% isoflurane. Anesthesia was initiated in an induction chamber.

The data were processed in Living Image® Software Version 2.50 (Xenogen Corporation). Regions of interest (ROIs) of equivalent size were manually drawn over the right lateral tumor-bearing hemisphere (TBH) of the tumor-bearing mice, the right lateral non-tumor-bearing hemisphere (NTBH) of the non-tumor-bearing control mice, and a normal (muscle) region defined on the hindlimb of the individual mice. The average total photon flux (photons/second) $\pm$ standard deviation for each group were plotted as a function of time post-injection to generate time-activity curves and to monitor the clearance rate and

accumulation of the TSPO-targeted and non-targeted probes. In addition, three contrast ratios were calculated to further determine tumor specificity: (1) TBH/M defined as the average ratio of the fluorescence intensity of the TBH relative to the normal muscle tissue (M) in the same mouse, (2) TSPO-Targeted/Free defined as the ratio of the average fluorescence intensity of the TBHs of the mice injected with NIR-conPK11195 relative to the TBHs of the mice injected with free NIR dye, and (3) TBH/NTBH defined as the ratio of the average fluorescence intensity of the TBHs of the tumor-bearing mice relative to the NTBHS of the non-tumor-bearing control mice.

Statistical Analysis

Statistical significance was determined using a one-way analysis of variance (ANOVA) test and the Holm-Sidak method for pairwise multiple comparisons with an overall significance level of 0.05 (SigmaStat v3.10).

RESULTS AND DISCUSSION

To evaluate the feasibility of TSPO-targeted molecular imaging as a potential approach to label breast cancer cells and metastases in the brain, human metastatic breast adenocarcinoma (MDA-MB-231) cells were stereotactically implanted into the basal ganglia of athymic nu/nu mice. Intracranial tumor-bearing mice and non-tumor-bearing control mice were subsequently imaged pre-injection and up to approximately 25 hours post-injection of either the NIR-labeled TSPO-targeted NIR-conPK11195 agent or free (unconjugated) NIR dye. Longitudinal images of intracranial MDA-MB-231 tumor-bearing mice (top panel) and non-tumor-bearing control mice (bottom panel) are displayed in Fig. (Fig. (1)), illustrating the distinct biodistribution, clearance, and accumulation profiles of the NIR-conPK11195 probe and free NIR dye. These false-colored NIR images are overlaid onto the corresponding white light images for anatomical reference and are displayed on the same intensity scale (photons/second/cm²/steradian) to enable a direct quantitative comparison between the biodistribution profiles of the TSPO-targeted and non-targeted fluorophores. This quantitation is further evaluated in terms of time-activity-curves (TACs) and three specific contrast ratios below. Prior to injection, the mice are essentially devoid of intrinsic NIR fluorescence as expected in the NIR wavelength range. Immediately post-injection, the NIR-conPK11195 and free NIR dye are fairly uniformly distributed throughout the mice. However, as early as four and a half hours post-injection, the NIR-conPK11195 appears to preferentially accumulate in the intracranial MDA-MB-231 TBHs, but not in the contralateral hemispheres or in the NTBHS of the control mice. In addition, the nontargeted NIR fluorophore has almost entirely cleared from both the intracranial tumor-bearing and normal mice, indicating that the enhancement is specific and due to the presence of the conPK11195 ligand. Over time, the TSPO-targeted and non-targeted agents continue to clear from the mice, resulting in significant labeling of the MDA-MB-231 cells by NIR-conPK11195 in the right lateral TBHs of the tumor-bearing mice, but not in the NTBHS of the control mice.

Fluorescence images acquired approximately 25 hours post-injection of NIR-conPK11195 or free NIR dye are displayed on a false-colored scale ranging from 0 to 100 × 10⁶

photons/sec/cm²/sr in Figs. (Fig. (2A–D)). To further highlight the tumor specificity of NIR-conPK11195, the images from Figs. (Fig. (2A–D)) are displayed on a smaller dynamic range (70 to 86 × 10⁶ photons/sec/cm²/sr) in Fig. (Fig. (2E–H)) and are overlaid onto the corresponding white light image for anatomical reference. Figs. (Fig. (2I–L)) illustrate the harvested brains from each of these mice (120 to 200 × 10⁶ photons/sec/cm²/sr). Increased fluorescence intensity is apparent in the right lateral TBH of the representative mouse's brain 25 hours post-injection of NIR-conPK11195 (Figs. Fig. (2A, E, I)), suggesting preferential uptake of the TSPO-targeted imaging agent by tumor tissue over the contralateral normal brain. Conversely, the non-tumor-bearing mouse injected with **NIR**-conPK11195 demonstrates fairly uniform residual fluorescence (Fig. Fig. (2C)) as well as a lack of fluorescence signal in the harvested brain (Fig. Fig. (2K)) approximately 25 hours post-injection. The evenly distributed fluorescence intensity in the normal tissues of both the tumor-bearing (Fig. Fig. (2A)) and non-tumor-bearing (Fig. Fig. (2C)) mice injected with NIR-conPK11195 is similar in magnitude and presumably results from the ubiquitous expression of TSPO, which has been shown to be significantly lower than that of tumor cells such as the MDA-MB-231 cell line [19]. Additionally, the free NIR dye has almost entirely cleared from both the tumorbearing (Fig. Fig. (2B, F, J)) and non-tumor-bearing (Fig. Fig. (2D, H, L)) mice injected with the non-targeted fluorophore, resulting in near baseline levels of **NIR** fluorescence. An example of the histological characterization of the tumor tissue within the brain by hematoxylin and eosin staining is shown in Fig. (Fig. (2M)), clearly indicating pathology typical of the type seen in breast cancer cells and metastases in the brain.

Overall, the images in Fig. (Fig. (2)) indicate preferential uptake of NIR-conPK11195 by the MDA-MB-231 tumor-bearing brain tissue over the contralateral (Fig. Fig. (2A, E, I)) and non-tumor-bearing normal brain (Fig. Fig. (2C, G, K)). In addition, the absence of fluorescence approximately 25 hours post-injection of free **NIR** dye in the tumor-bearing mice (Fig. Fig. (2B, F, J)) indicates that the uptake of NIR-conPK11195 is conPK11195-specific rather than the enhanced permeability and retention effect (EPR) often present in tumors [22]. This observation is in agreement with previous studies that have demonstrated TSPO-specificity of NIR-conPK11195 in *in vitro* MDA-MB-231 cells as well as an up to 7-fold contrast enhancement of *in vivo* MDA-MB-231 xenografts labeled with **NIR**-conPK11195 [18].

To quantitatively evaluate the *in vivo* utility and effectiveness of our TSPO-targeted NIR-conPK11195 agent to detect and monitor breast cancer cells in the brain, the fluorescence intensity of the right lateral TBH and normal muscle tissue regions were quantified in terms of total photon flux (photons/second) using equivalently sized regions of interest (ROIs). Fig. (3) displays the time activity curves (TAC) generated from these measurements, allowing quantitative evaluation of the clearance rates and accumulation of NIR-conPK11195 and free NIR dye *in vivo* [(A) all evaluated ROIs; (B) Tumor-bearing mice only; (C) TBH and equivalent NTBH regions from tumor-bearing and non-tumor-bearing control mice, respectively; (D) Contrast ratios corresponding to groups in B and C]. The TACs in Fig. (3) clearly demonstrate two distinct clearance profiles for the TSPO-targeted NIR-conPK11195 (red circle, solid line) and non-targeted free NIR dye (orange square, solid

line) in the intracranial tumor-bearing mice. More specifically, the time necessary to clear half of the maximum fluorescence intensity ($t_{1/2}$) in the TBHs of the intracranial tumor-bearing mice was approximately eight to ten hours for the NIR-conPK11195 and approximately three hours for the free NIR dye. These clearance rates are in agreement with the previously reported clearance rates of NIR-conPK11195 and free NIR dye in MDA-MB-231 xenografts on the murine hind limb [18]. Conversely, the NIR-conPK11195 cleared more rapidly from the equivalent NTBH regions of the control mice (Fig. 3 red circle, dashed line) with a $t_{1/2}$ of approximately five hours and the free NIR dye demonstrated an accelerated $t_{1/2}$ of approximately three hours in both the tumor-bearing (Fig. 3 orange square, solid line) and non-tumor-bearing mice (orange square, dashed line).

Fig. (3A, B) also illustrates the preferential accumulation of NIR-conPK11195 in the TBHs of the intracranial MDA-MB-231-bearing mice (red circle, solid line) relative to their normal muscle tissue (blue triangle, solid line) as well as the significant contrast enhancement compared to the free NIR dye in the TBH and muscle (solid lines, orange square and green diamond, respectively). The statistical significance of this contrast enhancement can be evaluated by calculating three important contrast ratios (Fig. 3D): (1) TBH/M defined as the average ratio of the fluorescence intensity of the TBH relative to the normal muscle tissue (M), (2) TSPO-Targeted/Free defined as the ratio of the average fluorescence intensity of the TBHs of the mice injected with NIR-conPK11195 relative to the TBHs of the mice injected with free NIR dye, and (3) TBH/NTBH defined as the ratio of the average fluorescence intensity of the TBHs of the tumor-bearing mice relative to the NTBHs of the non-tumor-bearing control mice. The plots in Figs. (3B and C) correspond to the two major columns in the table in Fig. (3D) for easier interpretation: (B) Tumor-bearing mice only (C) TBH and equivalent NTBH regions from tumor-bearing and non-tumor-bearing control mice, respectively.

After only four and a half hours post-injection, the fluorescence intensity of the TBHs of the mice injected with NIR-conPK11195 becomes significantly greater than the normal tissue with a 2.1-fold TBH/M contrast enhancement ($p < 0.005$; Fig. 3D, 1st column). This significant tumor-specific contrast persists, increasing to 2.4-fold at 24.5 hours post-injection ($p < 0.005$; Fig. 3D). Conversely, the TBH/M ratio of the mice injected with free NIR dye is not statistically significant at any time point post-injection (Fig. 3D, 2nd column). Additionally, the non-tumor-bearing control mice injected with either NIR-conPK11195 or free NIR dye demonstrate no appreciable accumulation of the TSPO-targeted or free dye in the equivalent NTBH regions; the tumor/normal ratios at all time points post-injection are approximately 1.1 to 1.3. Combined, these results indicate that the accumulation of NIR-conPK11195 in the TBHs is conPK11195-specific and not simply due to agent pooling and the EPR effect [22].

This tumor-specific contrast enhancement resulting from the preferential accumulation of NIR-conPK11195 is further demonstrated by comparing the TBH regions of the mice injected with NIR-conPK11195 with the TBH regions of mice injected with the free NIR dye (Fig. 3D, 3rd column). Immediately post-injection, the TBHs of the mice injected with NIR-conPK11195 and free NIR dye are equivalent (TSPO-targeted/free = 1.0). Over time, however, NIR-conPK11195 is taken up, presumably binds to TSPO, and is retained to a

significantly greater extent than the free NIR dye, resulting in significant 2.7- to 7.2-fold contrast enhancement ($p < 0.005$; Fig. 3D, 3rd column).

As a final measure of NIR-conPK11195 specificity, the TBHs of tumor-bearing mice were compared to the equivalent NTBH regions of the control mice (Fig. 3D, 4th and 5th column). A significant, approximately 3-fold contrast enhancement was seen in the TBHs relative to the NTBHs at all time points post-injection of NIR-conPK11195, as demonstrated by the TBH/NTBH ratio (Fig. 3D, 4th column). Conversely, the free NIR dye TBH/NTBH ratio remained statistically insignificant for the duration of the study (Fig. 3D, 5th column). These results suggest similar clearance rates and lack of accumulation of free NIR dye in both tumor-bearing and non-tumor-bearing mice as well as tumor-specific uptake of NIR-conPK11195 in the TBHs.

Although the NIR spectroscopic properties of NIR-conPK11195 enables increased tissue penetration over visible fluorescent agents [23], the detection limits of this technique and the contrast threshold necessary to discriminate tumor tissues should be considered. All of the results discussed above were obtained five weeks post-implantation. At four weeks post-implantation, the fluorescence intensity of the TBHs was significantly greater (1.8-fold) than the normal tissue at 24 hours post-injection ($p < 0.01$), while the TBH/M ratio for free NIR dye-injected tumor-bearing mice remained statistically insignificant at all time points post-injection (1.0- to 1.3-fold; data not shown). However, at two and three weeks post-implantation, no contrast enhancement was appreciable in the TBHs relative to the normal tissues. Although the lack of observable signal is likely dictated by both the tumor size and location depth, we are encouraged by our ability to detect the MDA-MB-231 tumors in the mouse brain *in vivo* at both four and five weeks post-implantation. As optical instrumentation improves, *in vivo* imaging through the cranium and consequently performance of this probe should also improve. Nevertheless, NIR-conPK11195 appears to be valuable as a TSPO-targeted molecular imaging agent for studying breast cancer xenografts in the brain in preclinical mouse models as well as for drug discovery and therapeutic efficacy monitoring.

Furthermore, this research represents a potentially translatable imaging approach by simply replacing the optical tag with a different signaling moiety, such as a radiolabel. In fact, TSPO-targeted positron emission tomography (PET) agents have already been utilized in the clinic to image neuroinflammation through labeling of activated microglia [24–26] and human gliomas [27]. Though specificity may be a slight limitation of this agent, we speculate that it may be possible to extend the applicability of NIR-conPK11195 for imaging of other tumor types and neurodegenerative diseases and believe that it will be advantageous to detect the overall presence of disease. However, definitive evidence for the use of NIR-conPK11195 for additional indications will require evaluating various animal models of disease. In addition, recent evidence has suggested variable binding of some TSPO-targeted agents in normal brain [28]. Though the investigators found ¹¹C- PK11195 to be the exception, it may be necessary to elucidate the binding characteristics in normal and diseased brain.

Moving forward, we envision that this agent may also have utility for intraoperative tumor localization, enabling more aggressive tumor resections with less chance of inducing detrimental neurological deficits to the normal brain tissue. Current surgical navigation systems that are used to intraoperatively guide tumor resections are generally based on preoperative CT or MR images [29]. However, these systems suffer from spatial inaccuracies that result from intraoperative brain deformation and registration errors, and are limited by the sensitivity of the preoperative imaging modality [29, 30]. The use of intraoperative computed tomography (iCT), intraoperative ultrasound (iUS), and intraoperative magnetic resonance imaging (iMRI) have also been explored, but are limited by additional radiation exposure to the patient and neurosurgeons (iCT), echogenic signals resulting from the contused brain, irrigation fluid and peritumoral edema (iUS), or incompatibility with standard operating equipment, limited patient access and expense (iMRI) [29, 31, 32]. In an effort to overcome some of these limitations, a number of optical techniques have been investigated for intraoperative brain tumor demarcation including those based on endogenous spectral differences between diseased and normal tissue [33–36] as well as those which utilize exogenous contrast agents [37–46]. Although each of these methods has demonstrated some success and possess unique advantages over the clinically accepted intraoperative techniques, the use of a targeted NIR molecular imaging agent, such as NIR-conPK11195, should overcome some of the major limitations of these methods including brief temporal contrast (fluorescein-based agents and indocyanine green), potential toxicity (Qdots), and predominant uptake by tumor-infiltrating macrophages and microglia rather than tumor cells (Cy5.5-CLIO and Qdots). Furthermore, the TSPO-specificity of this agent should provide additional functional information regarding the tumor phenotype, aggressiveness and potentially stage.

CONCLUSIONS

In this study, we provide the first demonstration of the use of TSpO imaging for detecting breast cancer cells in the murine brain *in vivo* using a non-invasive, longitudinal molecular imaging approach and a TSPO-targeted NIR probe in a preclinical animal model. Quantitative TACs illustrate the distinct clearance profiles for the TSPO-targeted NIR-conPK11195 and unconjugated NIR dye. Contrast ratios derived from these TACs clearly illustrate preferential labeling of the TBHs by NIR-conPK11195 and not from the free NIR dye as well as a lack of signal enhancement in the NTBHS by either NIR-conPK11195 or free NIR dye. More specifically, the significant 2.4-fold contrast enhancement in the TBHs relative to the normal muscle tissue 24h post-injection of NIR-conPK11195 coupled with the 2.7- to 7.2-fold TSPO-targeted/free contrast enhancement in the TBHs indicate that NIR-conPK11195 preferentially accumulates in the TBHs and is conPK11195-specific. Overall, this study suggests the applicability of TSPO-targeted molecular imaging to study breast cancer brain metastases using a NIR-labeled conPK11195 agent in an intracranial MDA-MB-231 breast cancer model. We envision that this agent may also prove useful for intraoperative tumor demarcation for surgical resection guidance. Furthermore, this preclinical study provides some impetus for exploring the use of TSPO-specific PET or single photon emission computed tomography (SPECT) radiotracers for imaging breast cancer brain metastases in the clinical setting, particularly in light of the fact that [^{11}C]-

labeled TSPO probes such as [^{11}C]- PK 11195 have been successfully used to image human gliomas.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Masakazu Shiota for graciously assisting with the tail vein injections, and LI-COR Biosciences, Inc. for providing a portion of the dye used in this study. This research was supported by the National Science Foundation (NSF BES-0323281) and National Institutes of Health (NIH P20 GM072048-02).

REFERENCES

- [1]. American Cancer Society. Cancer Facts and Figures 2012.
- [2]. Cheng X, Hung MC. Breast cancer brain metastases. *Cancer Metastasis Rev* 2007; 26: 635–43. [PubMed: 17717635]
- [3]. Lin NU, Bellon JR, Winer EP. CNS metastases in breast cancer. *J Clin Oncol* 2004; 22: 3608–17. [PubMed: 15337811]
- [4]. Palmieri D, Smith QR, Lockman PR, et al. Brain metastases of breast cancer. *Breast Dis* 2006; 26: 139–47. [PubMed: 17473372]
- [5]. Rugo HS. The importance of distant metastases in hormone- sensitive breast cancer. *Breast* 2008; 17 Suppl 1: S3–8. [PubMed: 18279764]
- [6]. Weil RJ, Palmieri DC, Bronder JL, Stark AM, Steeg PS. Breast cancer metastasis to the central nervous system. *Am J Pathol* 2005; 167: 913–20. [PubMed: 16192626]
- [7]. Sharma M, Abraham J. CNS metastasis in primary breast cancer. *Expert Rev Anticancer Ther* 2007; 7: 1561–6. [PubMed: 18020924]
- [8]. Schaller BJ, Cornelius JF, Sandu N, Buchfelder M. Molecular imaging of brain tumors personal experience and review of the literature. *Curr Mol Med* 2008; 8: 711–26. [PubMed: 19075670]
- [9]. Heyn C, Ronald JA, Mackenzie LT, MacDonald IC, Chambers AF, Rutt BK, Foster PJ. In vivo magnetic resonance imaging of single cells in mouse brain with optical validation. *Magn Reson Med* 2006; 55: 23–9. [PubMed: 16342157]
- [10]. Heyn C, Ronald JA, Ramadan SS, et al. In vivo MRI of cancer cell fate at the single-cell level in a mouse model of breast cancer metastasis to the brain. *Magn Reson Med* 2006; 56: 1001–10. [PubMed: 17029229]
- [11]. Song HT, Jordan EK, Lewis BK, Gold E, Liu W, Frank JA. Quantitative T(2)* imaging of metastatic human breast cancer to brain in the nude rat at 3 T. *NMR Biomed* 2011; 24: 325–34. [PubMed: 20949637]
- [12]. Song HT, Jordan EK, Lewis BK, et al. Rat model of metastatic breast cancer monitored by MRI at 3 tesla and bioluminescence imaging with histological correlation. *J Transl Med* 2009; 7: 88. [PubMed: 19840404]
- [13]. Bai M, Wyatt SK, Han Z, Papadopoulos V, Bornhop DJ. A novel conjugable translocator protein ligand labeled with a fluorescence dye for in vitro imaging. *Bioconjug Chem* 2007; 18: 1118–22. [PubMed: 17552492]
- [14]. Deane NG, Manning HC, Foutch AC, et al. Targeted imaging of colonic tumors in smad3–/– mice discriminates cancer and inflammation. *Mol Cancer Res* 2007; 5: 341–9. [PubMed: 17426249]
- [15]. Manning HC, Goebel T, Marx JN, Bornhop DJ. Facile, efficient conjugation of a trifunctional lanthanide chelate to a peripheral benzodiazepine receptor ligand. *Org Lett* 2002; 4: 1075–8. [PubMed: 11922786]
- [16]. Manning HC, Goebel T, Thompson RC, Price RR, Lee H, Bornhop DJ. Targeted molecular imaging agents for cellular- scale bimodal imaging. *Bioconjug Chem* 2004; 15: 1488–95. [PubMed: 15546219]
- [17]. Manning HC, Smith SM, Sexton M, et al. A peripheral benzodiazepine receptor targeted agent for in vitro imaging and screening. *Bioconjug Chem* 2006; 17: 735–40. [PubMed: 16704212]

- [18]. Wyatt SK, Manning HC, Bai M, et al. Molecular imaging of the translocator protein (TSPO) in a pre-clinical model of breast cancer. *Mol Imaging Biol* 2010; 12: 349–58. [PubMed: 19949989]
- [19]. Han Z, Slack RS, Li W, Papadopoulos V Expression of peripheral benzodiazepine receptor (PBR) in human tumors: relationship to breast, colorectal, and prostate tumor progression. *J Recept Signal Transduct Res* 2003; 23: 225–38. [PubMed: 14626449]
- [20]. Hardwick M, Fertikh D, Cuity M, Li H, Vidic B, Papadopoulos V. Peripheral-type benzodiazepine receptor (PBR) in human breast cancer: correlation of breast cancer cell aggressive phenotype with PBR expression, nuclear localization, and PBR-mediated cell proliferation and nuclear transport of cholesterol. *Cancer Res* 1999; 59: 831–42. [PubMed: 10029072]
- [21]. Maaser K, Grabowski P, Sutter AP, et al. Overexpression of the peripheral benzodiazepine receptor is a relevant prognostic factor in stage III colorectal cancer. *Clin Cancer Res* 2002; 8: 3205–9. [PubMed: 12374690]
- [22]. Iyer AK, Khaled G, Fang J, Maeda H. Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discov Today* 2006; 11: 812–8. [PubMed: 16935749]
- [23]. Weissleder R A clearer vision for in vivo imaging. *Nat Biotechnol* 2001; 19: 316–7. [PubMed: 11283581]
- [24]. Banati RB, Newcombe J, Gunn RN, et al. The peripheral benzodiazepine binding site in the brain in multiple sclerosis: quantitative in vivo imaging of microglia as a measure of disease activity. *Brain* 2000; 123 (Pt 11): 2321–37. [PubMed: 11050032]
- [25]. Venneti S, Lopresti BJ, Wiley CA. The peripheral benzodiazepine receptor (Translocator protein 18kDa) in microglia: from pathology to imaging. *Prog Neurobiol* 2006; 80: 308–22. [PubMed: 17156911]
- [26]. Yasuno F, Ota M, Kosaka J, et al. Increased binding of peripheral benzodiazepine receptor in Alzheimer's disease measured by positron emission tomography with [¹¹C]DAA 1106. *Biol Psychiatry* 2008; 64: 835–41. [PubMed: 18514164]
- [27]. Junck L, Olson JM, Ciliax BJ, et al. PET imaging of human gliomas with ligands for the peripheral benzodiazepine binding site. *Ann Neurol* 1989; 26: 752–8. [PubMed: 2557794]
- [28]. Owen DR, Gunn RN, Rabiner EA, et al. Mixed-affinity binding in humans with 18-kDa translocator protein ligands. *J Nucl Med* 2011; 52: 24–32. [PubMed: 21149489]
- [29]. Peters TM. Image-guidance for surgical procedures. *Phys Med Biol* 2006; 51: R505–40. [PubMed: 16825730]
- [30]. Sinha TK, Miga MI, Cash DM, Weil RJ Intraoperative cortical surface characterization using laser range scanning: preliminary results. *Neurosurgery* 2006; 59: ONS368–76; discussion ONS376–67. [PubMed: 17041506]
- [31]. LeRoux PD, Berger MS, Ojemann GA, Wang K, Mack LA Correlation of intraoperative ultrasound tumor volumes and margins with preoperative computerized tomography scans. An intraoperative method to enhance tumor resection. *J Neurosurg* 1989; 71: 691–8. [PubMed: 2681567]
- [32]. Sosna J, Barth MM, Kruskal JB, Kane RA. Intraoperative sonography for neurosurgery. *J Ultrasound Med* 2005; 24: 1671–82. [PubMed: 16301724]
- [33]. Gebhart SC, Majumder SK, Mahadevan-Jansen A. Comparison of spectral variation from spectroscopy to spectral imaging. *Appl Opt* 2007; 46: 1343–60. [PubMed: 17318255]
- [34]. Lin WC, Toms SA, Johnson M, Jansen ED, Mahadevan-Jansen A. in vivo brain tumor demarcation using optical spectroscopy. *Photochem Photobiol* 2001; 73: 396–402. [PubMed: 11332035]
- [35]. Lin WC, Toms SA, Motamedi M, Jansen ED, Mahadevan-Jansen A Brain tumor demarcation using optical spectroscopy; an in vitro study. *J Biomed Opt* 2000; 5: 214–20. [PubMed: 10938786]
- [36]. Toms SA, Lin WC, Weil RJ, Johnson MD, Jansen ED, Mahadevan-Jansen A. Intraoperative optical spectroscopy identifies infiltrating glioma margins with high sensitivity. *Neurosurgery* 2005; 57: 382–91; discussion 382–91. [PubMed: 16234690]
- [37]. Haglund MM, Berger MS, Hochman DW. Enhanced optical imaging of human gliomas and tumor margins. *Neurosurgery* 1996; 38: 308–17. [PubMed: 8869058]

- [38]. Haglund MM, Hochman DW, Spence AM, Berger MS. Enhanced optical imaging of rat gliomas and tumor margins. *Neurosurgery* 1994; 35: 930–40; discussion 940–31. [PubMed: 7838344]
- [39]. Ichioka T, Miyatake S, Asai N, et al. Enhanced detection of malignant glioma xenograft by fluorescein-human serum albumin conjugate. *J Neurooncol* 2004; 67: 47–52. [PubMed: 15072447]
- [40]. Kircher MF, Mahmood U, King RS, Weissleder R, Josephson L. A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation. *Cancer Res* 2003; 63: 8122–5. [PubMed: 14678964]
- [41]. Moore GE. Fluorescein as an agent in the differentiation of normal and malignant tissues. *Science* 1947; 106: 130–1.
- [42]. Popescu MA, Toms SA. In vivo optical imaging using quantum dots for the management of brain tumors. *Expert Rev Mol Diagn* 2006; 6: 879–90. [PubMed: 17140375]
- [43]. Stummer W, Novotny A, Stepp H, Goetz C, Bise K, Reulen HJ. Fluorescence-guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. *J Neurosurg* 2000; 93: 1003–13. [PubMed: 11117842]
- [44]. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol* 2006; 7: 392–401. [PubMed: 16648043]
- [45]. Stummer W, Stocker S, Wagner S, et al. Intraoperative detection of malignant gliomas by 5-aminolevulinic acid- induced porphyrin fluorescence. *Neurosurgery* 1998; 42: 518–25; discussion 525–16. [PubMed: 9526986]
- [46]. Trehin R, Figueiredo JL, Pittet MJ, Weissleder R, Josephson L, Mahmood U. Fluorescent nanoparticle uptake for brain tumor visualization. *Neoplasia* 2006; 8: 302–11. [PubMed: 16756722]

Fig. (1).

Longitudinal imaging of intracranial MDA-MB-231-bearing mice (top) and non-tumor-bearing control mice (bottom) illustrates the biodistribution and clearance of NIR-conPK11195 and free NIR dye, resulting in the preferential labeling of the tumor-bearing hemisphere (TBH) by the TSPO-targeted NIR-conPK11195, but not free NIR dye. False-colored NIR images were overlaid onto the corresponding white light images and displayed on the same photons/second/cm²/steradian scale. TBH = tumor-bearing hemisphere; NTBH = non-tumor-bearing hemisphere.

Fig. (2).

NIR-conPK11195 preferentially labels MDA-MB-231 cells grown in the right lateral brain hemisphere of athymic nu/nu mice. (A) Intracranial MDA-MB-231-bearing mouse injected with NIR-conPK11195, (B) Intracranial MDA-MB-231-bearing mouse injected with free NIR dye, (C) Non-tumor-bearing mouse injected with NIR-conPK11195, (D) Non-tumor-bearing mouse injected with free NIR dye, (E-H) Images from A-D, respectively, displayed on a smaller dynamic range fluorescence intensity scale to further highlight the tumor-specificity of NIR-conPK11195, (I-L) Harvested brains from mice A-D, respectively, (M) Hematoxylin and eosin stain of an intracranial MDA-MB-231-bearing brain approximately 25 hours post-injection of NIR-conPK11195.

Fig. (3).

Quantitative time-activity curves (TACs) and contrast ratios illustrate the distinct clearance profiles of NIR-conPK11195 and free NIR dye *in vivo* as well as the significant preferential accumulation of the TSPO-targeted NIR-conPK11195 in the intracranial MDA-MB-231-bearing brain hemisphere. **(A)** TACs for NIR-conPK11195 and free NIR dye in the tumor-bearing hemisphere (TBH) and normal muscle regions of intracranial MDA-MB-231-bearing mice and the equivalent non-tumor-bearing hemisphere (NTBH) and normal muscle regions of non-tumor-bearing mice; **(B)** TACs for NIR-conPK11195 and free NIR dye in the TBH and normal muscle regions of intracranial MDA-MB-231-bearing mice only; **(C)** TACs for NIR-conPK11195 and free NIR dye in the TBH and equivalent NTBH of intracranial MDA-MB-231-bearing mice and non-tumor-bearing mice, respectively; **(D)** Contrast ratios at each time point that correspond to the groups represented in **B** and **C**. TB = tumor-bearing mice; TBH = tumor-bearing hemisphere; C = control, non-tumor-bearing mice; NTBH = non-tumor-bearing hemisphere; M = muscle; NIR-conPK = NIR-conPK11195; Free = free (unconjugated) NIR dye. * $p < 0.05$.