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Detection of breast cancer microcalcification using ^{99m}Tc-MDP SPECT or Osteosense 750EX FMT imaging

Dayo D. Felix [a], John C. Gore [a,b,c,d,f], Thomas E. Yankeelov [a,b,c,d,e], Todd E. Peterson [a,b,c], Stephanie Barnes [a,b], Jennifer Whisenant [a,b], Jared Weis [a,b], Sepideh Shoukouhi [a,b], John Virostko [a,b], Michael Nickels [a,b], J. Oliver McIntyre [a,b,e], Melinda Sanders [g], Vandana Abramson [h], Mohammed N. Tantawy [a,b,*]

[a] Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA
[b] Department of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA
[c] Department of Physics Astronomy, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA
[d] Department of Biomedical Engineering, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA
[e] Department of Molecular physiology and Biophysics, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA
[f] Department of Cancer Biology, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA
[g] Department of Pathology, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA
[h] Department of Hematology/Oncology, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA

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ABSTRACT

Background: In previous work, we demonstrated the presence of hydroxyapetite (type II microcalcification), HAP, in triple negative MDA-MB-231 breast cancer cells. We used ^{18}F-NaF to detect these types of cancers in mouse models as the free fluorine, $^{18}F^-$, binds to HAP similar to bone uptake. In this work, we investigate other bone targeting agents and techniques including ^{99m}Tc-MDP SPECT and Osteosense 750EX FMT imaging as alternatives for breast cancer diagnosis via targeting HAP within the tumor microenvironment.

Methods: Thirteen mice were injected subcutaneously in the right flank with 10^6 MDA-MB-231 cells. When the tumor size reached ~0.6 cm^3, mice ($n = 9$) were injected with ~37 MBq of ^{99m}Tc-MDP intravenously and then imaged one hour later in a NanoSPECT/CT or injected intravenously with 4 nmol/g of Osetosense 750EX and imaged 24 hours later in an FMT ($n = 4$). The imaging probe concentration in the tumor was compared to that of muscle. Following SPECT imaging, the tumors were harvested, sectioned into 10 μm slices, and underwent autoradiography or von Kossa staining to correlate ^{99m}Tc-MDP binding with HAP distribution within the tumor. The SPECT images were normalized to the injected dose and regions-of-interest (ROIs) were drawn around bone, tumor, and muscle to obtain the radiotracer concentration in these regions in units of percent injected dose per unit volume. ROIs were drawn around bone and tumor in the FMT images as no FMT signal was observed in normal muscle.

Results: Uptake of ^{99m}Tc-MDP was observed in the bone and tumor with little or no uptake in the muscle with concentrations of 11.34 ± 1.46 (mean ± SD), 2.22 ± 0.95, and 0.05 ± 0.04 %ID/cc, respectively. Uptake of Osteosense 750EX was also observed in the bone and tumor with concentrations of 0.35 ± 0.07 (mean ± SD) and 0.04 ± 0.01 picomoles, respectively. No FMT signal was observed in the normal muscle. There was no significant difference in the bone-to-tumor ratio between the two modalities (5.1 ± 2.3 for SPECT and 8.8 ± 2.2 for FMT) indicating that there is little difference in tumor uptake between these two agents.

Conclusion: This study provides evidence of the accessibility of HAP within the breast tumor microenvironment as an *in vivo* imaging target for bone-seeking agents. SPECT imaging using ^{99m}Tc-MDP can be rapidly translated to the clinic. FMT imaging using Osteosense 750EX is not currently approved for clinical use and is limited to animal research.

1. Introduction

Mammograms often reveal small bone-like calcium deposits associated with breast cancer [1–5]. While there are many theories on the origin of these calcium deposits, more and more evidence in the past years has indicated that they could be the end result of a microcalcification process that begins inside the cell and then migrates to the microenvironment surrounding the tumors [6,7]. There are two types of microcalcifications: type I contain calcium oxalate, (CO), CaC_2O_4, generally associated with benign disease or non-invasive lobular carcinoma *in situ*, and type II contain calcium phosphates in the form of carbonated calcium hydroxyapatite (HAP), $Ca_{10}(PO_4)_6OH_2$, and are mainly associated with malignancy [1,5–12]. β-Glycerophosphate (βG) is hydrolyzed to glycerol and inorganic phosphate (Pi) on the malignant cell surface by alkaline phosphatase (ALP). Pi is then transported into the cell by the type II family of Na-Pi

* Corresponding author at: Department of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Vanderbilt University, 1161 21st Ave South, MCN AA-1105, Nashville, TN 37232, USA. Tel.: +1 615 343 4795; fax: +1 615 322 0734.
E-mail address: mohammed.n.tantawy@vanderblt.edu (M.N. Tantawy).

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cotransporters where Pi then combines with calcium to produce HAP crystals. Finally, HAP leaves the cells and settles into the extracellular matrix [6,7]. Therefore HAP microcalcifications may be a sign of precancerous cells or early breast cancer including ductal carcinoma *in situ* (DCIS) [13].

HAP is also the basic component of normal skeletal bone [14,15]. Single Photon Emission Computed Tomography (SPECT) using [^{99m}Tc] methylene diphosphonate (^{99m}Tc-MDP) and Fluorescence Molecular Tomography (FMT) using Osteosense have been used extensively (and exclusively) for bone imaging. The uptake of ^{99m}Tc-MDP in bone is due to both chemical adsorption of ^{99m}Tc-MDP onto the surface of the HAP in bone and incorporation into the crystalline structure of HAP [16,17]. Osteosense is a synthetic phosphonate derivative that binds to HAP [18].

We have previously reported on the presence of HAP in the MDA-MB-231 triple negative breast cell line and provided evidence for the use of ^{18}F-NaF positron emission tomography (PET) imaging for detecting MDA-MB-231 tumors in mouse models [19]. Upon administration, $^{18}F^-$ dissociates from the sodium ion in the blood and binds to HAP within the tumor microenvironment in a manner similar to bone uptake. In this work, we expand on our previous work and investigate the use of other bone-targeting agents including 99mTc-MDP and Osteosense 750EX in a multimodality approach to image MDA-MB-231 tumors in mouse models of breast cancer via targeting the HAP within the tumor microenvironment. We then compare the results of this work to ^{18}F-NaF PET results from our previous study.

2. Methods

All animal studies were approved by the Vanderbilt University Committee on Institutional Animal Care prior to conducting the experiments.

2.1. Tumor model

The mouse models were created as described in previous work [19]. Briefly, the MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin (Invitrogen, Carlsbad, CA) at 37 °C in a humidified, 5% CO_2 incubator. Approximately 10 million MDA-MB-231 cells in a 1:2 ratio of matrigel and DMEM were injected subcutaneously in the right hind limb of four to five week old female Foxn1 nu/nu mice ($n = 13$). Imaging sessions were conducted when the tumor size reached ~0.6 cm^3 (as measured by calipers).

2.2. SPECT/CT imaging

Mice ($n = 9$) were anesthetized with 2% isofluorane and injected with ~37 MBq/0.2 mL ^{99m}Tc-MDP intravenously. The mice were positioned in a NanosSPECT/CT (Bioscan, Washington DC) at approximately 1 hour post radiotracer administration. A nine pinhole aperture, each with a pinhole diameter of 1.4 mm, was used on each of the four camera heads to allow for maximum sensitivity. Twenty-four projections at 60 seconds per projection were acquired for a total scan time of ~30 min. SPECT acquisitions were reconstructed via the manufacturers HiSPECT software using Ordered Subsets Expectation Maximization (OSEM) iterative reconstruction algorithm with 9 iterations and 4 subsets. CT images were conducted at an x-ray beam intensity of 90 mAs with an x-ray energy of 45 kVp. The SPECT/CT images were reconstructed into $176 \times 176 \times 300$ slices with a voxel size of $0.2 \times 0.2 \times 0.2$ mm^3.

2.3. Autoradiography and staining

Upon completion of the SPECT/CT imaging sessions, the mice were euthanized and the tumors were harvested. Sections (10 μm) of the tumor and muscle (from the contralateral hind limb) were imaged in a Beta MicroImager (Biospace Labs, France) or stained with von Kossa to correlate ^{9m}Tc-MDP distribution sites with staining results. The stained slide and the autoradiography slide were subsequent sections to allow for better visual correlation of the two slides.

2.4. FMT imaging

Mice ($n = 4$) were injected with 4 nmol/g Osteosense 750EX (Perkin Elmer Inc, Boston, MA) intravenously. This dose was chosen based on the recommendations of the manufacturer. Approximately 24 hours later, the mice were anesthetized with 2% isofluorane and positioned in an FMT 2500 (Perkin Elmer Inc., Boston, MA). Scanning was performed using the 750EX channel. The entire mouse, except head and tail, were in the field-of-view. The scan lasted approximately 20 min. The images were reconstructed using the on-board TrueQuant software into a $512 \times 512 \times 13$ array with a voxel size of $0.163 \times 0.163 \times 1$ mm^3.

2.5. Additional CT imaging

One of the mice had a tumor with size >1500 mm^3. It was euthanized without SPECT imaging and then imaged in a microCT 50 (SCANCO, Switzerland) at an x-ray beam intensity of ~1 kAs and an x-ray peak voltage of 45 kVp. The scan lasted approximately 4 hours. The image was reconstructed at a resolution of $0.45 \times 0.45 \times 0.45$ mm^3.

2.6. Data analysis

A-SPECT/CT: Regions-of-interest (ROIs) were manually drawn around the entire tumor, bone (hips + spine), and the muscle of the contralateral hind limb in the CT images for each mouse using the medical imaging analysis tool AMIDE[20] and superimposed on the SPECT images. The total radiotracer concentration within the tumor was compared to the mean radiotracer concentration of the bone and muscle using one way ANOVA followed by a Tukey's test using Prisim version 4.03 (Graphpad Software, Inc., La Jolla, Ca, USA). The statistical significance threshold was considered to be $p < 0.05$.

B-FMT: Cylindrical ROIs were drawn around the tumor and part of the spine using the onboard TrueQuant software.

3. Results

3.1. SPECT/CT imaging

Tumor volumes using the CT images were estimated to be ~0.6 ± 0.15 cm^3. Uptake of ^{99m}Tc-MDP was limited to the bone, liver, and tumor with little or no uptake observed in the muscle as shown in Fig. 1. Bone and tumor uptake of ^{99m}Tc-MDP was clearly visible in the SPECT images with little or no uptake in the muscle and normal tissue. The average radiotracer concentration, normalized to the injected dose (percent injected dose per unit volume, %ID/cc), was 2.22 ± 0.95 (mean ± SD), 0.05 ± 0.04, and 11.34 ± 1.46 in the tumor, muscle, and bone, respectively (please see Fig. 2). The concentrations in any of those regions were significantly different from any of the other two regions ($p < 0.0001$), i.e. tumor vs muscle, tumor vs bone, and bone vs muscle.

3.2. Autoradiography and staining

Autoradiography signal was well correlated with positive von Kossa stains (red/brown) found in the tumor sections. No ^{99m}TC-MDP signal nor positive von Kossa stains were found in the muscle as shown in Fig. 3.

3.3. FMT imaging

We observed Osteosense 750EX uptake in the bone (0.35 ± 0.07 mean ± SD picomoles) and tumor (0.043 ± 0.01) with the uptake in the bone, as expected, significantly higher than that of the tumor