An Imaging Biomarker of Early Treatment Response in Prostate Cancer that Has Metastasized to the Bone

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Abstract
Prostate cancer ranks as the most common lethal malignancy diagnosed and the second leading cause of cancer mortality in American men. Although high response rates are achieved using androgen blockade as first-line therapy, most men progress toward hormone-refractory prostate cancer. Systemic chemotherapies have been shown to improve clinical outcome in hormone refractory prostate cancer patients; however, they are not curative. Due to the high incidence of bone involvement in hormone-refractory prostate cancer, assessment of treatment response in metastatic prostate cancer to the bone remains a major clinical need. In this current study, we investigated the feasibility of using the functional diffusion map (fDM) as an imaging biomarker for assessing early treatment response in a preclinical model of metastatic prostate cancer. The fDM biomarker requires a pretreatment and midtreatment magnetic resonance imaging diffusion map, which is used to quantify spatially distinct therapeutic-induced changes in the Brownian motion (or diffusion) of water within tumor tissue. Because water within tumor cells is in a restricted environment relative to extracellular water, loss of cell membrane integrity and cellular density during therapy will be detected by fDM as an increase in diffusion. Regions of significantly increased diffusion values were detected early using fDM in docetaxel-treated versus untreated metastatic prostate bone tumors at 7 days post treatment initiation (P < 0.05), indicating loss of tumor cell viability. Validation of fDM results was accomplished by histologic analysis of excised tissue. Results from this study show the capability of fDM as a biomarker for detection of bone cancer treatment efficacy, thus warranting clinical evaluation.

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Introduction
According to the American Cancer Society, there were an estimated 232,090 new cases of prostate cancer diagnosed in 2005 in the United States with ~30,350 men succumbing to metastatic disease. This ranks prostate cancer as the most common lethal malignancy diagnosed in American men and the second leading cause of male cancer mortality. Although first-line therapy with androgen deprivation has a high response rate, most men inevitably become hormone refractory. Systemic chemotherapy using docetaxel has been established as a standard of care for hormone-refractory prostate cancer as phase III trials have shown prolonged survival (1). However, there is no curative therapy, further necessitating the need for development of novel therapies to combat this disease. Impinging on the development of novel therapeutics for metastatic prostate cancer is the inability to readily assess tumor response. Although prostate-specific antigen has been explored as a potential response measure, with a 50% drop in serum levels indicating an antitumor effect, the use of prostate-specific antigen end points as a reliable surrogate for survival has not been prospectively validated in phase III clinical trials. In metastatic prostate cancer, bone represents the most common site of involvement in which ~90% of advanced prostate cancer patients are afflicted with bone lesions during the course of disease (2). Moreover, currently available imaging strategies for assessing treatment response of bone lesions are lacking, which further confounds the treatment of metastatic prostate cancer.

In the treatment of metastatic prostate cancer, imaging technologies have been solely used for detecting metastatic lesions because no current imaging strategy is able to decisively assess tumor response to therapy. This is highlighted by the fact that current standards for assessing bone tumor response fail to meet the needs of oncologists (3, 4). Moreover, the Response Evaluation Criteria in Solid Tumors system explicitly deems bone disease to be “nonmeasurable.” Given the clear need for assessing treatment response of bone tumors, the current study aims to determine the feasibility of diffusion magnetic resonance imaging (MRI) and functional diffusion mapping (fDM) to serve as reliable response biomarkers.

In brief, diffusion MRI exploits the Brownian motion of water within tissues as a biomarker that correlates with cellularity (5, 6). Conceptually, effective treatment of a tumor, leading to disruption of cellular integrity and subsequent loss in overall cellular density, results in an increase in diffusion values detectable by diffusion MRI (7). As such, diffusion MRI has received great interest within the arena of oncology following an initial study showing that this technique provided early quantitative assessment of tumor response to therapy (8). More importantly, the recently described fDM technique, which uses a unique method for interpreting diffusion MRI data, has shown promise as an early biomarker for treatment response in glioma patients. In these studies, the fDM approach was able to provide early and reliable stratification of patient populations into response categories defined by traditional outcome measures (9, 10). However, the use of imaging for assessing treatment response of solid bone lesions has remained elusive in clinical care.

In this current study, fDM was evaluated as a biomarker for assessing response of bone lesions using a preclinical model of metastatic prostate cancer consisting of human PC-3 cells stably

Conflict of interest: A. Rehemtulla, T.L. Chenevert, and B.D. Ross have a financial interest in the use of the underlying technologies.

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expressing the firefly luciferase gene (PC3Luc). The use of in vivo bioluminescence detection facilitated the identification of focal metastatic lesions within the animal, which would subsequently be entered into the magnetic resonance studies. In numerous studies, the use of optical reporters, such as bioluminescence and fluorescence, afforded the ability to monitor disease progression and treatment response as the amount of optical signal is proportionate to the amount of disease within each animal (11–14). As such, the luciferase reporter in this preclinical model provided a dynamic indicator of treatment response throughout the study. In addition, bone tumor response to docetaxel therapy was also monitored using standard measurements of response such as growth kinetics and histopathology, which were correlated with diffusion MRI and fDM findings. Our results showed that fDM analysis provided early indication of positive tumor response to treatment, which preceded discernible differences in tumor growth kinetics between treated and control groups. Moreover, histologic assessment of excised bone lesions was spatially correlated with fDM within regions showing positive response to therapy as well as regions that did not respond. Taken together, these results show the potential application of the fDM approach as a sensitive biomarker for assessing early changes in tumor response of lesions residing in the bone.

Materials and Methods

Cell lines. PC3 cells were transfected with a luciferase-encoding plasmid retroviral construct using Fugene 6 (Roche Applied Science, Indianapolis, IN) per manufacturer’s instructions. Wild-type PC3 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum whereas transfected cells (PC3Luc) were maintained in selection media supplemented with 200 μg/mL G418 (Invitrogen, Carlsbad, CA).

Mice. Male severe combined immunodeficient mice (Charles River Laboratories, Wilmington, MA) were housed in specific pathogen-free rooms at The University of Michigan Association for Assessment and Accreditation of Laboratory Animal Care International accredited facilities. For implantation of PC3Luc cells, mice were anesthetized with 1.75% isofluorane/air anesthesia and 2 × 10⁶ cells in 100 μL of sterile Dulbecco’s PBS lacking Ca²⁺ and Mg²⁺ were administered into the left ventricle of the heart.

Bioluminescence detection. Approximately 4 to 5 weeks after PC3Luc implantation, animals were screened using bioluminescence to select subjects exhibiting metastatic disease. Subsequently, animals were divided into untreated control (n = 11) and docetaxel-treated (n = 9; 40 mg/kg/wk × 3) groups. Bioluminescence studies were initiated before treatment for baseline values and done throughout the experiment. For these studies, mice were anesthetized with a 2% isofluorane/air mixture and given a single i.p. dose of 150 mg/kg D-luciferin (Promega, Madison, WI) in normal saline. Animals were then reanesthetized using 2% isofluorane/air mixture ~8 min post administration of D-luciferin and images were acquired ~10 to 12 min post administration of D-luciferin. For photon counting, a charge-coupled device camera system (Xenogen, Alameda, CA) with a nose-cone isofluorane delivery system and heated stage for maintaining body temperature was used. Results were analyzed using Living Image software provided with the Xenogen imaging system. Signal intensity was quantified as the sum of all detected photon counts within a uniform region of interest manually placed during data postprocessing.

MRI. A subset of control (n = 5) and docetaxel-treated (n = 7) animals from the bioluminescence screening study, which had evidence of focal metastases within the leg, were entered into the magnetic resonance studies. For MRI examination, mice were anesthetized with a 2% isofluorane/air mixture and maintained at 37°C, using a heated water bed, inside a 1.5-T Varian magnetic resonance scanner (120-mm clear horizontal bore; Varian, Palo Alto, CA) and a double-tuned volume radiofrequency coil. A trace diffusion-weighted multislice spin echo sequence was used to acquire 15 slices with two different diffusion weightings (b1, 184 s/mm²; b2, 1,106 s/mm²; slice thickness, 0.5 mm; image matrix, 64 × 128; field of view, 15 × 15 mm; echo time, 40 ms; repetition time, 3.5 s). The z-gradient first moment was zeroed to reduce the dominant source of motion artifact as well as a 32-point navigator echo was prepended to each phase-encode echo. The phase deviation of each navigator echo relative to their mean was subtracted from the respective image echoes before the phase-encode Fourier transform. Images were acquired before treatment and subsequently twice per week. The low b-factor images were essentially T2 weighted to allow tumor volume measurements. The tumor boundary was manually defined on each slice and then integrated across slices to provide a volume estimate.

Image registration and fDM. An important part of fDM analysis is the registration of posttreatment ADC maps to baseline pretreatment ADC maps. Image registration was done using an automated linear affine coregistration algorithm to maximize mutual information between the two temporally distinct three-dimensional data sets (15). Following registration and segmentation of voxels within the tumor both at baseline and on day 4, fDM statistics were calculated (i-Response, Cedara Software, Mississauga, Ontario Canada). First, ADC values of voxels posttherapy were plotted as a function of baseline ADC values. These voxels were then further segmented into three regions based on the upper and lower thresholds of ADC change. Tumor voxels with an ADC increase above the upper threshold were depicted as red, whereas voxels that had decreased below the lower threshold were depicted as blue. All other voxels that did not change significantly were depicted as green.

Histopathology. Approximately 2 weeks post treatment initiation, selected animals from the control (n = 2) and docetaxel-treated (n = 2) groups were sacrificed immediately after acquisition of magnetic resonance data and tumor-bearing legs were harvested for staining. Tissue sections

Figure 1. Bioluminescence imaging of control and treated animals. A, representative images from control (top) and treated (bottom) animals from weeks 1, 2, 3, and 4. B, points, mean normalized photons from control (n = 11) and treated (n = 8) groups on days 0, 7, 14, and 21 post treatment initiation, bars, SE.
(5-μm thickness) were stained with H&E using routine protocols. The histopathology results were then compared with fDM using distinguishable landmarks as well as dimensional analysis.

Results and Discussion

For the current study, a previously reported model of metastatic prostate cancer with a high incidence of bone involvement was used (12). As previously mentioned, this disease model was generated through intracardiac injection of human PC3Luc tumor cells stably expressing firefly luciferase into severe combined immunodeficient mice. Approximately 4 weeks post injection of PC3Luc cells, bioluminescence detection was used to monitor differences in luciferase activity between control and treated groups. Serial images from representative control and treated animals are provided in Fig. 1A. For the control animal, luciferase activity continued to markedly increase from week 1 to 4. However, in the representative treated animal, despite an initial increase, luciferase activity remained relatively unchanged especially when comparing week 4 with week 1. The mean photon counts from control (n = 11) and treated (n = 9) groups were calculated and plotted in Fig. 1B showing a continuous signal increase in control animals, whereas docetaxel-treated animals remained stable throughout the experiment. These results indicated that docetaxel therapy had an effect in stunting disease progression as compared with controls.

Animals that presented metastatic lesions in the leg region, based on bioluminescence detection, were entered into the magnetic resonance studies. To better illustrate the fDM approach, a representation of a tumor residing in the bone with a pseudo-fDM overlay generated from coregistration of diffusion MRI data pretreatment and posttreatment. After treatment, regional changes of ADC are plotted on the image to provide a visual representation of tumor response. Green voxels, areas of the lesion that did not respond to therapy such that a significant change in ADC was not detected. In the event of a positive therapeutic response, loss of cellularity within responsive regions results in a significant increase in ADC (>0.4 × 10−3 m²/s, red voxels). B, fDM analysis from a representative control animal at days 7 and 11 post initiation of PBS treatment. Top, fDMs from a representative control animal are provided for qualitative assessment of overall changes in tumor ADC. Bottom, scatter plots were also generated and the percentage of increased ADC was determined to be 1.2% and 2.5% on days 7 and 11, respectively. C, fDM analysis from a representative treated animal at days 7, 11, 14, and 18 post initiation of docetaxel therapy. Top, fDMs for the representative treated animal. Bottom, scatter plots were also generated and the percentage of increased ADC was determined to be 4.0% (day 7), 13.1% (day 11), 30.0% (day 14), and 53.7% (day 18). D, fDM analysis from a representative treated animal. Top, fDMs for the representative treated animal. Bottom, scatter plots were also generated and the percentage of increased ADC was determined to be 4.0% (day 7), 13.1% (day 11), 30.0% (day 14), and 53.7% (day 18). D, points, mean percentage of increased ADC from the control (n = 5) and treated (n = 7) groups as a function of time post initiation of therapy; bars, SE. E, points, mean normalized volumes from control (n = 5) and treated (n = 7) groups as a function of time; bars, SE.
for qualitative assessment of overall changes in tumor ADC. Corresponding scatter plots (Fig. 2B, bottom) were also generated and the percentage of increased ADC was determined to be 1.2% and 2.5% on days 7 and 11, respectively. fDM analysis from a representative treated animal at day 7, 11, 14, and 18 post-initiation of docetaxel therapy was also done as shown in Fig. 2C. fDMs (Fig. 2C, top) for the representative treated animal revealed an increase in regions of red voxels over time, and corresponding scatter plots (Fig. 2C, bottom) determined the regions of increased ADC to be 4.0% (day 7), 13.1% (day 11), 30.0% (day 14), and 53.7% (day 18). Mean changes in tumor diffusion, as determined by fDM, from control (n = 5) and treated (n = 7) groups were plotted in Fig. 2D. Control animals revealed insignificant change in diffusion values whereas treated animals revealed progressively increasing fDM diffusion changes (red regions) over the 3-week treatment period. A statistically significant difference in fDM values between control and treated animals was achieved as early as 7 days post treatment (P < 0.05; Fig. 2D). MRI determination of tumor volumes revealed continued growth of control tumors throughout the experiment whereas treated tumors did not (Fig. 2E), which correlates with our fDM findings and indicates that docetaxel therapy was efficacious. Moreover, differences in tumor volumes between the two groups reached significance at day 11 post treatment initiation (P < 0.05), much later than was observed by fDM, suggesting that fDM could serve as an early biomarker for treatment response.

Validation of the fDM findings was accomplished through spatially correlating fDM with H&E-stained histologic sections from control (Fig. 3A) and treated (Fig. 3B) tumors excised 14 days following treatment initiation (16). For the control tumor, fDM data revealed minimal changes in tumor diffusion values, which corresponded to histologic observations of a highly dense and cellular morphology (Fig. 3A). A macroscopic cross-sectional view of the leg from a treated animal (Fig. 3B) showed a much more heterogeneous morphology in which regions of high and low cellularity were apparent throughout the tumor mass. The fDM image corresponding to the proximal region of the lesion with reduced cellularity showed (Fig. 3C, bottom left) that a significant portion of the tumor mass, as depicted by the red voxels, responded to therapy. In contrast, the fDM image corresponding to the distal (Fig. 3C, bottom right) region showed a lack of significant treatment response consistent with histologic findings. Microscopic images (Fig. 3C, top left) from a region corresponding to an area of red voxels revealed a high degree of cellular disintegration. For comparison, a microscopic image from a region of green voxels in the distal fDM image (Fig. 3C, top right) revealed a relatively dense and highly cellular tumor region. Due to the lack of a nonresponsive tumor to serve as a negative control, the possibility exists that the observed fDM changes could be due to secondary effects of docetaxel therapy rather than a distinct antitumor effect. However, our previous results have clearly and reliably shown that a drastic increase in tumor water diffusion values is directly correlated with an underlying decrease in cellular density resulting from a positive therapeutic response (16, 17). Moreover, previous studies have also shown that diffusion MRI was able to ascertain the loss of antitumor effect and emergence of drug resistance when resistant tumors exhibited decreased diffusion change on further treatment with an initially effective therapy (18, 19). As such, the observed fDM changes in this prostate cancer model seem to be consistent with the anticipated antitumor effects of docetaxel therapy, which correlated both temporally and spatially with the histopathologic findings. Taken together, these histologic and diffusion findings show that the fDM imaging biomarker is capable of detecting spatially distinct changes in tumor cellularity in response to therapeutic intervention.

Results of this study highlight the sensitivity and the quantitative aspects of the fDM approach as an imaging biomarker, as well as its ability to provide high spatial and temporal resolution for assessment of therapeutic response. However, metastatic hormone refractory prostate cancer commonly presents with diffuse skeletal involvement (multiple sites of bone metastases). In this current figure 3. Standard measures of treatment response. A, top, a representative bone lesion 14 d post injection of PBS was immediately extracted after diffusion MRI acquisition for histologic analysis by H&E staining. Bottom, comparative analysis of fDM and histology revealed that the green region corresponded to an area of high cellularity and density. B, the fDM image (bottom left) from the proximal region of the tumor suggests that a region of the lesion responded to therapy, which correlated with the corresponding H&E-stained region (top left), revealing low cellular density. FDM analysis of the distal tumor region (bottom right) suggests a lack of treatment response as little change in ADC was detected. Comparative analysis of the fDM image and corresponding H&E-stained section (top right) reveals a relatively dense cellular morphology. C, H&E staining of the femur section taken 14 d post treatment initiation revealed a bone lesion heterogeneous in density with the proximal region appearing to be less dense as compared with the distal region.
study, a single lesion was monitored for evaluating the fDM approach. Future efforts evaluating the use of whole body diffusion MRI imaging to follow multiple metastatic lesions over time are warranted (20).

In summary, this study provides the foundation to develop an imaging strategy that has both preclinical and clinical applications. The use of the fDM approach could greatly enhance the preclinical development of novel therapies by allowing for rapid and quantitative noninvasive assessment of drug efficacy in vivo. More importantly, the clinical translation of this technique could greatly affect treatment planning in which early detection of an ineffective therapy would provide an opportunity to switch to an alternative therapy in a timelier manner thereby providing for the prospect of individualized patient care.

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References