Improved Magnetic Resonance Imaging Detection of Prostate Cancer in a Transgenic Mouse Model

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ABSTRACT

Transgenic mouse models of prostate cancer provide an opportunity to conduct genetic tests of the molecular mechanisms underlying initiation and progression of tumorigenesis. They also allow assessment of the effects of various pharmacological interventions. However, one limitation that has impeded full exploitation of these models is the lack of in vivo imaging procedures of sufficient sensitivity and resolution to detect and follow tumors at early stages of growth. We have addressed this problem through the use of diffusion-weighted magnetic resonance imaging (DWI). A transgenic mouse model (CR2-TAg) of prostate cancer was used to show that DWI can detect tumors <1 mm in diameter. Markedly enhanced DWI contrast results from a 2-fold difference in apparent diffusion coefficient between benign and malignant prostatic tissue (P < 0.00001). Clinical application of DWI may offer advantages over current T2-weighted magnetic resonance imaging methods.

INTRODUCTION

Cancer of the prostate is the fourth most frequent cancer of men overall in the world (1). It is also the second most common cause of cancer morbidity and mortality in American males, currently accounting for ∼30% of new cancer cases (2). Mouse models of prostate cancer not only permit direct genetic tests to be conducted on the contributions of selected factors to initiation or progression of tumorigenesis but also allow the effects of various pharmacological interventions to be assayed in genetically well-defined hosts. One key to realizing the full potential of such mouse models is development of noninvasive imaging strategies with sufficient sensitivity and resolution to detect and monitor the early growth and therapeutic responses of tumors in an organ that normally has dimensions of a few millimeters.

MRI represents one approach for addressing this issue. Currently, tumor image contrast is developed through the use of T2 relaxation. Prostate cancer is typically seen as an island of low signal intensity (indicative of a shorter T2 relaxation time constant for tumor) enclosed by high signal intensity (longer T2) from surrounding benign peripheral tissue (3, 4). However, MRI typically requires a long spin echo time (TE) to obtain sufficient cancer-to-normal prostate contrast because of a limited relaxation time constant differential between benign and neoplastic tissue (4). The result is decreased signal-to-noise ratio and loss of image definition, which in turn increases interobserver variance. T2-weighted MRI provides sufficient sensitivity for the detection and monitoring of large prostate tumors in mice, i.e., those with diameters greater than several millimeters (5).

An alternative to T2-weighted MRI is to develop image contrast through the as yet poorly understood sensitivity of “apparent diffusivity” (tissue water incoherent displacement over distances of ∼1–20 μm) to pathological processes. DWI has been used in both clinical and research settings for detecting cerebral (6–9), as well as cancer-related pathologies (10–14). We hypothesized that the extensive branching ductal structure of the normal prostate compared with the highly restricted intracellular and interstitial spaces encountered in prostate cancers would produce a substantial ADC differential and, thus, high image contrast.

Recently, a transgenic mouse (CR2-TAg) model of metastatic prostate cancer, originating from neuroendocrine cells, has been developed to investigate the significance of neuroendocrine differentiation in human prostate cancer (15). In this model, SV40 large T antigen was expressed, under control of transcriptional regulatory elements from the mouse cryptdin-2 (CR2) gene, in a subset of neuroendocrine cells. CR2-TAg transgene expression begins at 7–8 weeks of age. The result is a neoplastic transformation of cells within a week, with subsequent rapid and stereotyped progression to invasive prostate cancer by 12–16 weeks of age. Thus, CR2-TAg mice provided a system to compare and contrast T2- and diffusion-weighted MRI. Our results indicate that the differential in ADC of normal prostate and prostate tumor in vivo is much greater than that in T2, enabling detection of prostate tumors considerably <1 mm in diameter.

MATERIALS AND METHODS

Preparation of Mice for MRI. All procedures were carried out according to protocols approved by Washington University Animal Studies Committee and were in compliance with the Animal Welfare Act and NIH Guide for the Care and Use of Laboratory Animals. CR2-TAg transgenic mice (n = 8) and their age-matched normal littermates (n = 10) were anesthetized with a halothane and oxygen mixture (5% for induction, 1% for maintenance) using a nose cone. A 2-cm inner diameter quadrature radio frequency coil was used for 3H data acquisition. All studies were performed with an Oxford Instruments-200/330 (4.7 tesla, 33-cm clear bore) magnet equipped with a 16-cm inner diameter, actively shielded magnetic field gradient coil and power supply (18 G/cm, 400 μs rise time). The magnet and gradient coil and power supply were interfaced with a Varian UNITY INOVA console controlled by a Sun Microsystems Ultra-170 Sparc workstation.

Histological Studies. At the conclusion of some MRI examinations, mice were perfused with 4% paraformaldehyde in PBS. Bladder, urethra, and prostate were removed en bloc and embedded in paraffin. Serial sections (6-μm thickness) of the entire prostate were then prepared. Every 10th section was stained with H&E and examined by light microscopy.

MRI

Transverse (or Spin-Spin) Relaxation Time Constant (T2) Measurements. A multislice, multi spin-echo imaging sequence was used for T2 determinations. Imaging parameters were as follows: 3 s TR, 30 ms TE; 27 ms TE increment of the echo chain, four echoes per echo chain; 0.3-mm slice thickness.
Fig. 1. T2- and diffusion-weighted 1H magnetic resonance images of prostate cancer in a CR2-TAg transgenic mouse. Differential T2-weighted MRI (A and B, TE = 30 and 57 ms, respectively) and diffusion-weighted MRI (C, b = 0.764 m²/μm², TE = 36 ms) from a tumor-bearing CR2-TAg transgenic mouse. Position of the tumor is indicated by a black arrow and normal prostate by an arrowhead. The tumor versus normal prostate tissue contrast is improved significantly in the diffusion-weighted image. Displayed field of view in all images is 15 mm × 15 mm. Scale bars = 1 mm.

ADC Measurements. ADC data were obtained using a conventional multislice spin-echo imaging sequence modified by adding single-axis Stejskal-Tanner diffusion sensitizing gradients. The pixel-by-pixel calculation of T2-weighted images according to Eq. [1],

\[ y(t) = M_0 \times e^{-\gamma T_2} \]

where \( y(t) \) is the pixel intensity at time \( t \) from individual images, \( M_0 \) is the expected pixel intensity at \( t = 0 \), and \( T_2 \) is as described above.

ADC Measurements. ADC data were obtained using a conventional multislice spin-echo imaging sequence modified by adding single-axis Stejskal-Tanner diffusion sensitizing gradients. The pixel-by-pixel calculation of ADC was performed according to Eq. [2] using two b values (0 and 0.764 m²/μm²).

\[ \ln \left( \frac{S_{b2}}{S_0} \right) = b \times ADC \]

where \( S_{b2} \) is the pixel intensity of the diffusion-weighted image with \( b = 0.764 \) m²/μm², and \( S_0 \) is the pixel intensity of the T2-weighted image (i.e., data acquired under the same acquisition conditions as \( S_{b2} \) except \( b \) = 0 m²/μm²). The diffusion-sensitizing factor, \( b \), is defined as Eq. [3]

\[ b = \gamma^2 \times G^2 \times \delta^2 \times (\Delta - \delta/3) \]

where \( \gamma \) is the gyromagnetic ratio, \( G \) is the amplitude of the diffusion-sensitizing gradient pulse, \( \delta \) is the diffusion gradient pulse duration, and \( \Delta \) is the time separation of the diffusion sensitizing gradient pulse pairs. Diffusion-weighted images were acquired using the following parameters: 2.5 s TR, 36 ms TE, 0.3-mm slice thickness; 3-cm field-of-view, and data matrix of 256 × 256. The pixel-by-pixel T2 calculation was performed following standard Fourier image reconstruction. This calculation used a standard Varian Image Browser two-parameter fitting routine on the pixel intensity values of standard Fourier images encompassing the tumor (regions of low signal intensity). Displayed field of view in all images is 15 mm × 15 mm. Scale bars = 1 mm.

To assess the effect of possible diffusion anisotropy on the DWI-derived tumor volume and shape, two diffusion sensitizing gradients were used along \( X \) and \( Y \) axes (slice selection and phase encoding directions of the imaging view) on one tumor-bearing mouse using the acquisition parameters described above. The MRI results were also compared with the results of postimaging histological analysis of the prostate from the same mouse.

Statistical Analysis. The T2 and ADC values of tissues in individual mice were determined by volume averaging of manually selected regions of interest across multislice images encompassing the tumor (regions of low signal intensity on the ADC map) or normal prostate from the T2 and ADC maps. The unpaired \( t \) test was used to compare the sample means of the individual T2 and ADC of tumors and normal prostate. Statistical significance was accepted at \( P < 0.05 \).

RESULTS

A CR2-TAg mouse with a single prostate tumor was used to collect MRI images with two different T2 weightings (TE = 30 and 57 ms) and an image acquired with diffusion weighting (\( b = 0.764 \) m²/μm², TE = 36 ms). The tumor appeared dark in both T2-weighted images (Fig. 1, A and B), whereas it was bright in the diffusion-weighted image (Fig. 1C). The tumor-to-prostate, contrast-to-noise ratios of the images, as derived by standard methods (16, 17), were 9.8 (panel A), 5.4 (panel B), and 17.2 (panel C).

Quantitative T2 and ADC maps of the prostate are shown in Fig. 2. The tumor appears as an island of low intensity surrounded by the high intensity of normal prostate tissue in both the T2 (panel A) and ADC maps (panel B). However, the differentiation of tumor versus normal prostate is much less apparent in the T2 map (see below).

Studies of a series of mice revealed that the normal prostate and tumor T2s were 45 ± 6 ms (mean ± SD, \( n = 10 \)) and 34 ± 10 ms (\( n = 6 \)), respectively (Fig. 2C). The ADCs were 1.47 ± 0.23 μm²/ms (\( n = 8 \)) and 0.47 ± 0.21 μm²/ms (\( n = 6 \)) for normal prostate and tumor, respectively (Fig. 2D). The normal prostate exhibits higher values of T2 and ADC compared with the values obtained from the tumor (T2, 132%; \( P = 0.054 \); ADC, 312%; \( P < 0.00001 \); note: derived confidence limits incorporated the different \( n \) values). The relative difference in ADC is substantially greater and statistically far more significant than in T2. Thus, in this mouse model, diffusion weighting offers improved contrast for differentiating normal prostate from prostate cancer.

A potential concern regarding the use of DWI is the effect of diffusion anisotropy (should it be present) on observed shape and size of the tumor. Such anisotropy was not anticipated a priori. Tumor size and shape derived from a T2-weighted image and from two different single-axis (X and Y) DWIs is displayed in Fig. 3 for a single mouse. The two different, single-axis DWIs and the T2-weighted image display similar tumor shape and size. More quantitatively, the volume-averaged single-axis ADC is not statistically different in tumor (ADCx = 0.33 ± 0.22 μm²/ms, ADCy = 0.41 ± 0.35 μm²/ms; \( P = 0.39 \)) or in normal prostate (ADCx = 1.2 ± 0.36 μm²/ms, ADCy = 0.5 ± 0.32 μm²/ms; \( P = 0.1 \)).

Fig. 2. Calculated T2 and ADC for tumor and normal prostate. The calculated T2 (A) and ADC (B) maps demonstrate the greater difference of ADC between tumor and normal prostate. The low intensity region indicated by the white arrow in both images is tumor. A linear scaling was used for image display. The mean T2 value (C) and ADC value (D) of the tumor and normal prostate were calculated from T2 and ADC maps from individual mice and are represented by the corresponding bar graph. Left-hatched bars, normal prostate; right-hatched bars, prostate tumor. Error bars, one SD from the mean.
and shape as that observed in the ADC map (Fig. 4), reveal two small tumors (0.8- and 0.3-mm in diameter) of similar size in the images. This is a good indication that diffusion anisotropy does not pose a problem in accurately defining tumor shape and size.

ADC_{e} = 1.3 \pm 0.52 \mu m^{2}/ms; P = 0.38), suggesting a lack of anisotropy. In a preliminary study where an individual mouse was examined using diffusion tensor imaging, no diffusion anisotropy of normal prostate was observed (data not shown). The volume-averaged relative anisotropy (18, 19) of the normal prostate was 0.04 \pm 0.02. A recent report on human prostate suggested that diffusion anisotropy is present (10). Although a quantitative anisotropy measurement was not performed, this latter finding raises the possibility that water displacement in mouse and human prostate may be different.

The quantitative ADC map shows that marked ADC contrast is present in early stage small prostate tumors (Fig. 4, A–C). Histological surveys of the same mouse prostate, harvested immediately after MRI, reveal two small tumors (0.8- and 0.3-mm in diameter) of similar size and shape as that observed in the ADC map (Fig. 4, D and E). Thus, small prostatic tumors maintain a marked ADC differential relative to normal prostate.

DISCUSSION

The prostates of CR2-TAg transgenic mice develop foci of increased cellularity with multiple mitotic and apoptotic features within a week of initiation of transgene expression (15). These foci resemble prostatic intraepithelial neoplasia, postulated to be a precursor to prostate cancer in humans. Small tumor nodules develop in these mice by 16 weeks of age. By 24 weeks, 100% of prostates contain large tumor nodules composed of solid masses of neoplastic cells (15).

The low signal intensity in T2-weighted MRI (shorter T2) of human prostate cancer may be related to the many tightly packed glandular elements with little central space for mucin or fluid storage. This tight glandular packing presents an increased restriction/hindrance to water displacement. Our MRI and histological studies of CR2-TAg mice support such a notion, with the histopathological features accounting for the observed diffusion-weighted hyper-intensity (lower ADC) of the tumor. The cellular transformations and structural changes in the prostate do not change tissue T2 characteristics to the extent that would allow the tissue contrast necessary to readily distinguish carcinoma from normal prostate in small tumors. The contrast between normal prostate and neoplastic foci achieved by heavy T2 weighting results in a diminished signal-to-noise ratio, i.e., signal intensity decreases with the long TE required to achieve such heavy T2 weighting. This results in degradation of image quality with loss of visualization of the internal zonal structures of the prostate. However, the same cellular and structural changes have a substantial impact on tissue water diffusion characteristics. The extent of the change in ADC was so striking that the contrast-to-noise ratio between tumor and normal prostate was improved to 2–3-fold that of T2-weighted images using a b value of 0.764 ms/µm² (Fig. 1). Our ADC measurements of tumor and prostate may, in principle, yield overestimated values because of perfusion weighting resulting from use of a b value of 0. However, we would expect tumor ADC to be overestimated more than that of normal prostate given the greater blood flow rate and vascular volume of tumor (20). The fact that the tumor ADC is 2-fold less than normal prostate suggests any perfusion contribution that may be present is not substantial.

In summary, we have demonstrated that tumor-to-prostate contrast is greatly enhanced in DWI of mice with genetically engineered prostate cancer. Although large prostate tumors were easily detected by T2-, spin density-, and diffusion-weighted MRI, diffusion-weighted imaging allowed detection of small tumors with relative ease. As in CR2-TAg mice, human prostate cancers are composed of tightly packed cellular clusters with little central space for storage of mucin or fluid. We believe that this shared feature of human and mouse prostate cancer may make DWI a useful tool for in vivo detection and staging of human cancers and for monitoring the efficacy of various therapeutic interventions (10). Diffusion MRI reports
from other laboratories examining prostate cancer lend support to this suggestion (21).

REFERENCES


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