Increases in apparent diffusion coefficient following therapy are correlated with region-specific cell death

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Introduction. The MRI-measured apparent diffusion coefficient of water (ADC_w) increases early in response to cancer therapies [1, 2]. Since cell death by apoptosis is a known early response to therapy which also involves cell shrinkage, it has been proposed that an increase in the extracellular volume fraction allows for increased unrestricted movement of water, thus causing an increase in ADC_w [3]. However, other modes of death can be initiated soon after beginning therapy. These other modes of death include mitotic catastrophe and necrosis, and also involve changes in the intra- and extracellular volume fractions [3]. We have examined whether MRI-measured ADC_w is altered in response to docetaxel therapy, which primarily induces cell death via these other mechanisms. Our data indicate that early and significant changes in ADC_w can occur in concert with mitotic catastrophe, suggesting that ADC_w may be a generalized measure of tumor response and that other mechanisms besides cell shrinkage may mediate the MR-measured changes.

Methods. MCF-7 human breast cancer cells (MCF-7 and MCF-7/D40) were implanted in the mammary fat pad of SCID mice and allowed to grow into tumors ranging in volume from 550 to 1400 mm^3. Diffusion-weighted MR images were generated one day before and two days after drug or sham treatment. Treatment was administered by intravenous injection. Docetaxel was administered in a single dose of 15 or 30 mg/kg. Animals were anesthetized, immobilized and the tumor placed in a home-built solenoid coil. Imaging was performed in a Bruker 4.7 T Biospec, with 14 G/cm self-shielded gradients. A radial scan diffusion-weighted MRI data acquisition method (DIFRAD) was used with fat suppression to generate axial slices covering the entire tumor volume [4]. Typical acquisition parameters were: TR = 1800 ms, TE = 52.00 ms, FOV = 3.00 x 3.00 cm, matrix size = 128 x 128, slice thickness = 2.00 mm (contiguous), Δ = 20.00 ms, δ = 7.00 ms, where δ and Δ represent duration and separation of diffusion gradients, respectively. Diffusion gradients were applied along the phase direction. This approach is sufficient due to low diffusion anisotropy in tumor xenografts (data not shown). At each slice location, images were obtained at four b values of 0, 200, 400 and 800 sec/mm^2 [$b = \gamma^2 G_d^2 \delta^2 (\Delta - \delta/3)$], where by increasing the gradient strength, G_d , and keeping Δ constant. γ is the gyromagnetic ratio for protons (42.58 MHz/T). Images were reconstructed from the radial data using magnitude filtered back projection, which minimizes artifacts due to motion [4]. Apparent diffusion coefficient (ADC_w) maps were generated by fitting the signal intensity of each pixel to a single exponential decay: $S = S_0 e^{-bADC_w}$ where S_0 is the signal intensity at b=0 sec/mm^2, and S is the signal intensity with diffusion weighting (Figure 1, A & B). Regions of interest (ROIs) (concentric circles) corresponding to peripheral, intermediate and center regions of the tumor (Figure 1, C) were traced on the center slice ADC_w map and ADC_w distribution histograms were generated. In some cases, the tumor was excised and sectioned for histology. Sections were H & E stained for determination of cell death morphologies and immunohistochemically stained for cleaved caspase 3 for determination of apoptosis. Histology images were analyzed using the analysis software Simple PCI [Compix, Inc. Imaging Systems].

Results. In the peripheral region, mitotic catastrophe as evidenced by aberrant mitoses was observed as the predominant mode of death 2 days post treatment (Figure 1, D). This was supported by a net increase in cell volume by image analysis (Table 1). A high baseline level of apoptosis was present pretreatment in the intermediate region which decreased post treatment. However, the predominant mode of death in the intermediate region was an increase in non-apoptotic small darkly-stained cells (possibly a form of necrotic death at early stages) which was accompanied by a net decrease in cell volume (Table 1). In the center, an increase in necrosis was observed as indicated by a decrease in cellularity (Table 1). Increases in mean ADC were regionally and dose dependent. Moderate ADC increases were observed in the periphery (~800 to 900). At the low dose, a moderate increase was observed in the intermediate region (669), while at the high dose a large increase was observed (1250). A large increase was observed in the center at the low dose (1035) while a relatively modest increase was observed at the high dose (240) (Table 1). These differences could be tumor specific as well as dose specific.

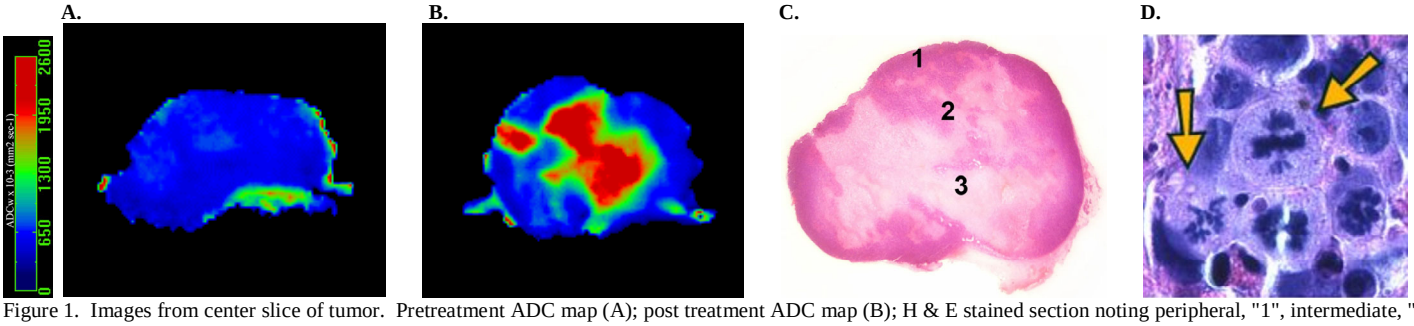

Figure 1. Images from center slice of tumor. Pretreatment ADC map (A); post treatment ADC map (B); H & E stained section noting peripheral, "1", intermediate, "2", and center "3" regions; and H & E stained peripheral region (100X) post treatment indicating enlarged cells undergoing mitotic catastrophe. Note: Units are ADC x 10^{-3} mm^2 sec^{-1}. ADC maps predominantly include tumor tissue, with superficial amounts of surrounding tissue. Only tumor tissue was included in ROIs.

response	Periphery	intermediate	center
mode of death	mitotic catastrophe predominates (net increase in cell volume), with slight increase in apoptosis.	increased non-apoptotic small darkly-stained cells (net decrease in cell volume), decreased apoptosis.	increase in necrosis (decrease in cellularity).
change in mean ADC (in sec mm^{-2})	sham treated: -11 15 mg/kg docetaxel: +897 30 mg/kg docetaxel: +822	sham treated: -17 15 mg/kg docetaxel: +669 30 mg/kg docetaxel: +1250	sham treated: +28 15 mg/kg docetaxel: +1035 30 mg/kg docetaxel: +240

Table 1. Regional cell death and ADC responses to docetaxel therapy in MCF-7 human breast cancer xenografts.

Discussion. Our results suggest that cell death responses other than apoptosis also play a role in the early tumor ADC_w response. These other mechanisms of death include mitotic catastrophe and necrosis. As seen in the tumor periphery, large cells arrested in mitosis and undergoing aberrant mitosis predominate in response to docetaxel therapy. This result suggests the possibility that the intracellular volume of these large anuclear cells may play a role in the observed increase in ADC_w. Decreased cellularity in the center of the tumor suggests that early (2 days) increases in diffusion can be due to lytic necrosis. The decrease in background levels of apoptosis in the intermediate region also supports the role of other cell death modalities in the overall ADC_w response.

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