Diffusion-weighted imaging detects early physiologic change of tumor following tamoxifen treatment in an MNU-induced breast-cancer rat model

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Purpose: Tamoxifen is the standard anti-ER therapeutic agent for breast cancer (approved by FDA) in the neoadjuvant as well as in adjuvant settings [**1, 2**]. Tamoxifen reduces tumor size for responding patients to facilitate breast conserving surgery without affecting survival, and it decreases the recurrence rate up to 50% regardless of menopausal status. However, tamoxifen has presented a wide range of sensitivity in individuals [**3**]. Only 60-70% patients with ERα-positive tumors benefit from hormonal therapy; 5% to 10% patients with ERα-negative tumors respond to tamoxifen, presumably via ERβ [**4**]. Because the characteristics of breast cancer vary among patients, it would be ideal to modify the therapeutic strategy for each patient. The purpose of this study was to determine the optimal imaging time point for early therapy assessment of tamoxifen using diffusion-weighted imaging (DWI) in a methylnitrosourea (MNU)-induced breast-cancer rat model, and further to explore the relationship between ERα (or ERβ) and tamoxifen therapeutic efficacy.

Methods: Two groups of Sprague-Dawley rats (n=15 for group 1; n=10 for group 2) were used. The rats (50 days old) were injected with MNU (50mg/kg body weight) through the jugular vein to induce spontaneous mammary tumors. When the tumors grew up to about 2 cm in diameter, T2W MRI and DWI were performed on days 0 (baseline), 3, and 7 using a 9.4T system (BioSpec, Bruker BioSpin) equipped with a surface coil as receiver. An MR imaging compatible small-animal respiratory gating device (SA instrument, Stony Brook, NY) was used during imaging. All rats were anesthetized with isoflurane (1-2%) during imaging. Two plastic bars were used to lift up the tumors, thus minimizing the motion artifacts. Anatomic imaging was acquired with a T2-weighted fast spin echo sequence (rapid acquisition with relaxation enhancement). The parameters were as follows: TR=3000ms, TE=34ms, rare factor=4, FOV=30×30mm, Matrix size=128×128, 20 slices, slice thickness=1mm. Diffusion weighted imaging was obtained with a diffusion weighted multi-slice 2D spin echo sequence. Four b factors (5, 300, 600, and 1000 s/mm^2) were applied in the x direction with the following parameters; TR=3501ms, TE=32ms, diffusion separation time=16ms, diffusion gradient duration=6ms, FOV=30×30mm, Matrix size=128×128, 5-7 slices to cover the tumor region, and slice thickness=1mm. The rats were treated with tamoxifen (10mg/kg diet) from day 0 after baseline imaging. After imaging on day 7, the treatment continued for group 1, while measuring tumor size twice weekly for another three weeks. Pearson correlation coefficients were obtained between the changes of intratumoral apparent diffusion coefficient (ADC) values and tumor volume. All animals of group 2 were euthanized on day 7 after imaging, and tumors (only assessed with MRI) were collected for histological analysis; Ki-67 and TUNEL staining were performed to quantify proliferating and apoptotic cell densities, respectively. ERα and ERβ densities were analyzed as well. The statistical correlation between the densities of the histologic markers and intratumoral ADC change was examined.

Results: DW images of mammary tumors were successfully obtained with minimal motion artifact (**Fig 1**). For group 1, the tumors were categorized into three sub-groups based on tamoxifen sensitivity; the periods for 50% volume reduction of sensitive, intermediate, and resistant tumors were 5.9±0.3 days (n=5), 12.3±1.5 days (n=6), and more than 28 days (n=4), respectively (**Fig 2a**). The mean ADC changes of sensitive, intermediate, and resistant tumors on day 3 after therapy initiation were 15.5±2.7%, 5.5±4.2%, and 7.5±2.6%, while those on day 7 were 27.7±6.1%, 12.7±5.6%, and 4.7±1.8%, respectively (**Fig 2b**). The ADC increase of the sensitive group was significantly higher than that of the resistant group over 7 days (p=0.014). The ADC changes on day 3 were significantly correlated with tumor-volume changes on days 3, 7, and 11 (p=0.0190, 0.0034, and 0.0020, respectively), while those on day 7 were significantly correlated with tumor-volume changes on days 7, 11, 14, 18, 21, and 25 (p=0.0006, 0.0001, 0.0027, 0.0056, 0.0348, and 0.0493, respectively). For group 2, animals were also categorized into three sub-groups according to the tumor reduction rate for 7 days after therapy initiation such as sensitive (>50%, n=4), intermediate (10%~50%, n=3), and resistant (<10%, n=3) groups. The tumor-volume reduction rates on day 7 of the three sub-groups were 61.5±4.5%, 27.0±6.2%, and -24.4±16.8%, respectively. The mean ADC changes of sensitive, intermediate, and resistant tumors on day 3 after therapy initiation were 11.6±3.2%, 2.4±6.0%, and -3.4±0.3%, while those on day 7 were 26.7±7.8%, 9.5±5.5%, and -8.0±2.6%, respectively. The ADC increase of the sensitive group was significantly higher than that of the resistant group over 7 days (p=0.0058). For group 2, ADC change on day 3 was significantly correlated with volume change on days 3 and 7 (p=0.0151 and p=0.0196, respectively); ADC change on day 7 was also significantly correlated with volume change on day 7 (p=0.0022). While there was no statistical difference in apoptotic cell and ER-alpha densities among the three groups, there was significant difference in Ki-67 cell density and ER-beta density between resistant and sensitive groups (p=0.0164 and p=0.0364, respectively). ADC change for 7 days was significantly correlated with apoptotic cell density, Ki-67 cell density, and ER-beta density (p=0.0320, 0.0052 and 0.0028, respectively), but not with ER-alpha density (p=0.8773).

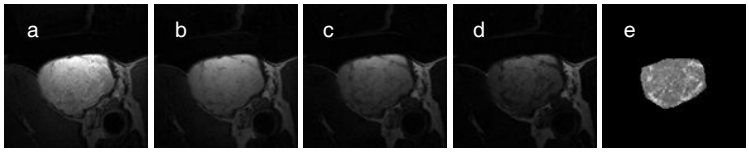

Figure 1. Representative diffusion-weighted images with (a) b=5, (b) b=300, (c) b=600, and (d) b=1000 sec/mm^2. (e) ADC map.

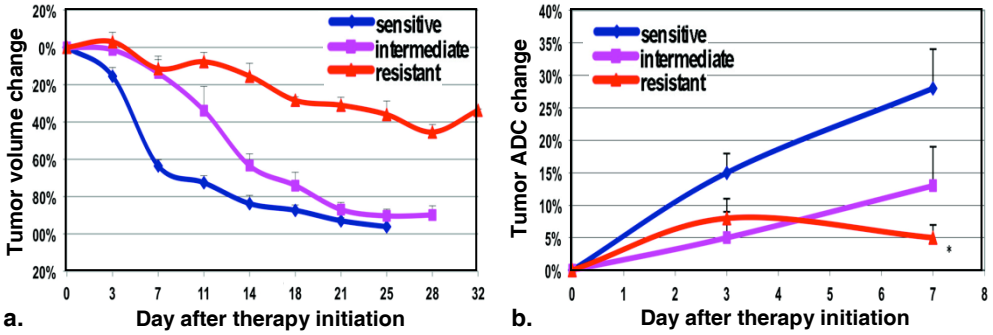

Figure 2. (a) Tumor volume change for 32 days, and (b) tumor ADC changes for 7 days after therapy initiation.

Conclusion: Our results suggest that the ADC change at 7 days after therapy initiation could be a reliable prognostic imaging biomarker to assess tamoxfen therapy for ER+ breast cancer patients. Of interest, the significant correlation between ADC change (or tumor-volume change) and ER-beta density suggests that ER-beta may play an important role for anti-tumor effect of tamoxifen.

REFs: [1] Miller, Br J Cancer 2006; 94:1333-1338. **[2]** Rose, Cancer Res 1992; 52:5386-5390. **[3]** Pritchard, Clin Cancer Res 2003; 9:460S-467S. **[4]** Sofia, Clin Cancer Res 2007; 13:1987-1994.