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Magnetization Transfer MRI Contrast May Correlate with Tissue Redox State in Prostate Cancer

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Abstract

Developing imaging biomarkers for non-invasive measurement of the tissue redox state is a key research area. Recently, we presented the first non-invasive MR imaging method that demonstrated the correlation between the endogenous chemical exchange saturation transfer (CEST) contrast and the tissue redox state. It is well known that the broadband magnetization transfer (MT) can occur via chemical exchange (CEST) and/or dipole–dipole interactions. The present study investigated if the broadband MT also correlated with the tissue redox state. The preliminary result for the prostate tumor xenografts indeed showed a significant correlation between the broadband MT contrast and the NADH redox ratio quantified with the optical redox scanning. In vivo MT contrast, once calibrated, may potentially serve as an imaging biomarker for tissue redox state.

Keywords

Oxidative stress; Redox state; Magnetization transfer; CEST; Prostate cancer; MRI

1 Introduction

It has been shown that the development of cancer cells and tumor progression is associated with altered intra- and extracellular redox regulation. Redox state alterations have been

associated with tumor malignancy [1, 2] and aggressiveness for breast cancer, melanoma, colon cancer, etc. [3–6].

Despite the importance of tissue redox state, so far there is no endogenous imaging contrast that can be used for the in vivo mapping of tissue redox status. Recently, we presented a non-invasive MR imaging method that demonstrated a significant correlation between the tissue redox state and the endogenous chemical exchange saturation transfer (CEST) contrast [7]. CEST MRI, on the basis of the proton exchange between metabolites and tissue bulk water, has been employed to non-invasively imaging tissue amide protons [8], liver glycogen [9], cartilage glycosaminoglycans [10], brain myo-inositol [11], and glutamate [12] with a spatial resolution down to sub-millimeters. In our recent study [7], CEST MRI has been employed to characterize intratumoral heterogeneity and to correlate with cancer redox states in xenografts of two breast cancer cell lines, MDA-MB-231 and MCF-7. We found a higher level of intratumoral CEST heterogeneity, calculated as the tumor rim-to-core ratio of CEST, in the aggressive (metastatic) MDA-MB-231 tumors than in the less aggressive (less metastatic or indolent) MCF-7 tumors. CEST contrasts from individual tumor rims and cores are linearly correlated with the NADH redox ratios quantified by the optical redox scanning where NADH is the reduced form of nicotinamide adenine dinucleotide.

It is well known that the broadband MT can occur via chemical exchange (CEST) and/or dipole–dipole interactions. The purpose of this study is to investigate whether broadband MT also correlates with the tissue redox state.

2 Methods

All in vivo animal studies were performed according to an Institutional Animal Care and Use Committee approved protocol at the University of Pennsylvania. The propagated cells of human prostate cancer of PC-3 and DU-145 were implanted subcutaneously into the upper thighs of athymic nude mice (~7 million cells/site). Tumor sizes were monitored once a week to obtain tumor volumes ($V = ab^2/2$, where a is the long axis and b is the short axis) [7]. After 5 weeks post implantation, mice bearing DU-145 and PC-3 prostate tumor xenografts ($n = 3$ for DU-145 and $n = 4$ for PC-3 tumors) were scanned at a Varian horizontal 9.4 T MRI scanner. Note that one PC-3 tumor was not included in the analysis due to image artifacts induced by severe B_1 inhomogeneity.

MTR spectra were collected from tumor central cross-sections using a custom sequence with a frequency selective rectangle saturation pulse ($B_1 = 250$ Hz, 1 s) followed by Fast Low-Angle Shot (FLASH) readout [7]. Other parameters were: flip angle = 15° , readout TR/TE = 6.2/2.9 ms, in-plane resolution = 0.27×0.27 mm². One saturation pulse followed with 64 segment acquisition was repeated every 4 s. The MTMRI contrastor MT ratio (MTR) was quantified as $(1 - MT_{on}/MT_{off}) \times 100\%$, where MT_{on} and MT_{off} referred to images acquired with saturation “on” at a specific frequency offset and “off” at 100 ppm, respectively. Except for Fig. 52.1a, all MTR shown and presented were for 20 ppm offset. MTR maps clearly desalinate solid tumor boundaries. After MRI, mice under anesthesia underwent snap-freezing procedures for tumor harvest. The excised frozen tumors were

embedded for multi-slice fluorescence imaging of NADH, oxidized flavoprotein (Fp), and NADH redox ratio (NADH/(Fp + NADH)) using the Chance redox scanner [3, 4]. The redox images of the sections closest to the MRI imaging lumen slices were chosen for the quantitative analysis and correlation with the MRI contrast.

Histograms of the MTR contrast and NADH redox ratio from each tumor were fit with two Gaussian distributions to separate the generally more oxidized tumor core and less oxidized rim [7]. One-tailed paired Student's t-test was performed for comparing the redox or MRI indices between tumor rim and core. Tumor core and rim values were pooled together for the correlation analysis between redox ratio and MTR, using the Excel data analysis regression tool. Statistical significance was attained when $P < 0.05$. Results are reported as mean $\pm$ standard error (SE).

3 Results

The average size of a prostate tumor 5 weeks after implantation for all mice was $164.3 \pm 20.1 \text{ mm}^3$ while the size of the DU-145 tumor was slightly larger than that of the PC-3 tumor (185.4 ± 31.2 vs. $143.1 \pm 24.7 \text{ mm}^3$, $P = 0.2$).

In vivo MTR spectra from prostate tumors are shown in Fig. 52.1a. Overall, reduced MT contrast was seen from DU-145 tumors compared to PC-3 tumors. Representative MTR maps (Fig. 52.1b) show typical hypointense tumor cores associated with DU-145 tumors, leading to higher levels of intratumoral inhomogeneity. As shown in Fig. 52.1c, NADH redox ratio maps of DU-145 and PC-3 tumors produced by ex vivo optical redox scanning present similar patterns as the corresponding in vivo MTR maps (Fig. 52.1b).

Figures 52.2a, c show the mean values of MTR and NADH redox ratios for each tumor. Overall, MTR and NADH redox ratio are higher in tumor rims than that in tumor cores (Fig. 52.2b, d, $P < 0.05$). Furthermore through a linear regression analysis pooling all data of tumor cores and rims, we found that the MTR contrasts were significantly correlated with the NADH redox ratios (Fig. 52.3, $R^2 = 0.77$, $P = 0.0002$). The 95 % confidence interval for the fitted linear coefficient was between 24.8 and 55.5 and that for the intercept between 0.1 and 13.8.

4 Discussion

This study validates and strengthens the hypothesis that the redox imbalance may affect magnetization transfer contrast and, furthermore, supports and shows consistency with our previous study [7]. The significant correlation between MT and NADH redox ratio found in this study indicates that in vivo MT contrast may potentially serve as an imaging biomarker for tissue redox status.

We hypothesize that the correlation between MT contrast and tissue redox status may be through proton/electron transfer in redox reactions, which are in a general form of $\text{RH}_2 + \text{NAD}^+ \leftrightarrow \text{R} + \text{NADH} + \text{H}^+$, where R is a substrate or tissue metabolite. Our previous study demonstrated that CEST MRI may probe proton transfer in redox reactions and that the reduced metabolites generally expressed higher CEST contrast than their oxidized

counterparts, which is likely due to the reception of mobile protons. As a result, higher CEST contrast corresponds to a more reduced redox state. Similarly, the MT effect from tissue metabolites may also be affected by their redox states. It is well known that MT can occur via chemical/proton exchange and/or dipole–dipole interactions. Metabolite oxidation leads to the loss of exchangeable protons or the mobile protons that generate MT contrast. In other words, MT MRI quantifies the steady-state redox states of all in vivo redox-sensitive metabolites, including small molecules, peptides, proteins and DNAs, whose redox states may be affected due to oxidative stress.

We could not rule out that there might be necrosis in some tumor core regions. In the clinic, tumor tissues are commonly classified into viable and necrotic regions, and there is not much interest in the necrotic regions from the pathologists in general. However, this binary tissue classification could be an over-simplified picture of tumor progression because cancer cells may still survive in necrotic regions, as shown previously in mouse xenografts of human breast cancer and melanomas [4, 13, 14]. These numerous viable cells in the tumor cores and their typically highly oxidized redox states could provide information on tumor metastatic potential [4, 13, 14]. Nevertheless, we still need to investigate whether this is the case for the prostate cancer models. Since MT contrast may include contributions from macromolecules in extracellular space, we also need to investigate whether the correlation between MT and redox state of tumor cores and rims still hold in the presence of known necrosis.

Compared to the CEST technique, broadband MT may have less sensitivity in the correlation with tissue redox state, particularly during the early phase of tumor growth. CEST contrast, but not MT, can differentiate the intratumoral core/rim heterogeneity of an aggressive breast cancer at small size [7].

A limitation of this study is that it is based on relatively small sample sizes. Although a significant correlation was found between NADH redox ratio and MTR contrast, larger sample size and longitudinal studies may improve the accuracy of the correlation between MT and tissue redox state. It is also worth noting that the MT contrast in vivo can be affected by many other factors, including tissue T_1 and T_2 relaxation times. We are currently investigating their possible contributions to the observed intratumoral MTR heterogeneity and their contributions to the correlation between MTR and redox ratios. Nevertheless, MT MRI for the characterization of tissue redox state requires calibration.

In conclusion, MT MRI contrast was found to significantly correlate to the prostate tumor NADH redox ratio measured by optical redox scanning. This correlation indicates that MT MRI may be sensitive to tissue redox state. Non-invasive MT MRI, once further confirmed, may have a great potential for clinical applications.

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Fig. 52.1.

(a) Z-spectra of DU-145 (*dashed line*) and PC-3 (*solid line*) prostate tumors. Data were averaged within entire tumor and across all tumors for each tumor line. (b) Representative MTR maps of a typical DU-145 tumor and a typical PC-3 tumor. (c) Optical redox scanning of representative DU-145 and PC-3 tumors, providing NADH, FP and NADH redox ratio maps

Fig. 52.2. MTR and NADH redox ratio of tumor core and rim from individual tumors (**a** and **c**, DU-145 and PC-3 tumors were grouped together) and the averaged values (**b** and **d**, * $P < 0.05$)

Fig. 52.3.

The MTR contrasts and the redox ratios from tumor cores and rims were significantly correlated (significance $P < 0.05$). The hollow black squares are derived from tumor cores and the solid ones are derived from tumor rims