Variation of ADC with Cell Cycle Phases: A Study Using Synchronized HL-60 Cells

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Introduction:

The factors that influence the apparent diffusion coefficient (ADC) of tissue water are of considerable interest but imperfectly understood. Although cell density is clearly important at long diffusion times, the values of ADC at short diffusion times may be influenced strongly by intracellular structure and changes. In particular, we postulated that ADC may change when cells undergo division, when there is massive synthesis and rearrangement of intercellular macromolecules, organelles, etc., as previously reported for relaxation times (1, 2). Moreover, proliferating tumors usually contain a much higher fraction of cells that are in active cell division phases, such as S (synthesis) and M (mitosis) phases, than normal tissues, so for a full understanding of the diffusion properties of tumors it is necessary to understand the changes that occur in cells in different phases (2, 3). Conventional pulsed gradient spin echo (PGSE) methods employ relatively long diffusion times, and thus cannot distinguish changes at sub-cellular dimensions from those at much larger scales. Oscillating gradient spin echo (OGSE) methods at moderately high frequency have the ability to probe much shorter diffusion times (5). Here we report OGSE studies of synchronized HL-60 cells at different phases.

Methods:

Six groups of synchronized HL-60 cell samples were prepared with thymidine treatment. Each group contains two types of samples. Type I (S phase) was treated for 24 hours, resulting in >80% cells were shown arrested in S phase. A second type (M phase) with >40% M/G2 phase was obtained by growing S phase cells for an additional 6 hours without thymidine. Both samples were pelleted and centrifuged to form ~10million cells pellets with similar cell densities.

All measurements were performed on a 7.0-T, 16-cm bore Varian INOVA spectrometer (Varian Inc. Palo Alto, CA) equipped with a Doty PFG/diffusion z-gradient coil (Doty Scientific Inc. Columbia, SC) with strength up to 1500G/cm. b values ranging from 500s/mm^2 to 10,000s/mm^2 were used in PGSE measurements. Gradient frequencies in OGSE measurements ranged from 50Hz to 2kHz, corresponding to characteristic diffusion length from ~4.5μm to ~0.7μm, which is much shorter than cell dimensions. This makes it possible to detect intracellular structure changes during cell division. Both diffusion methods utilized TR=3s, TE=64ms and gradient duration 20ms. All ADCs were obtained by a two-point fitting scheme (b=0, 1000 s/mm^2).

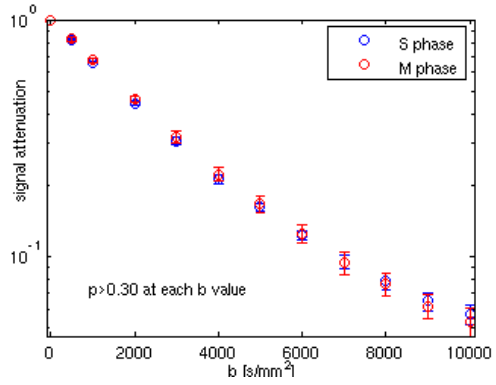

Fig.1 Signal attenuation obtained by PGSE measurements of two synchronized cell samples with b values up to 10,000s/mm^2.

Results:

Averaged ADCs of the two types of samples obtained by the PGSE method were not significantly different (0.42±0.01 and 0.42±0.02μm^2/ms). Moreover, the signal attenuation difference between the two samples was almost indistinguishable even with b values up to 10,000s/mm^2 (see Fig.1). On the contrary, ADCs obtained using the OGSE method are significantly different (>5% difference) especially at relatively higher frequencies (>500Hz, see Fig.2). Since samples were controlled to have the same cell densities, this implies that the intracellular structure changes that occur during cell division cycle affect the water ADC at ultra-short diffusion times.

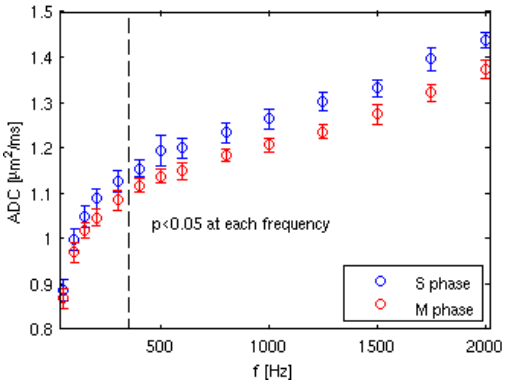

Fig.2 Dispersion curves (ADC vs f) of two types of synchronized cells. Error bars show standard deviations of all six samples.

Discussion and Conclusion:

Synchronized cell samples usually contain a mixture of cells in all cell cycle phases, so our results actually show an integrated effect of cell cycle phases. However, by measuring the amounts at each phase in different samples, the ADCs of cells at specific cell cycle phases can be estimated by a multiple regression fitting. Some previous studies have suggested that high b values may suppress extracellular water signals and hence increase the sensitivity of diffusion measurements to intracellular water (6). In the present work, no significant difference was observed with b value up to 10,000s/mm^2. This shows again that ADC values measured by the conventional PGSE method are dominated by cell density (4), which limits their ability to distinguish tissues with similar cell densities and sizes (7). The OGSE method used in the present work detects the intracellular structure changes during cell division cycle using short diffusion times, which provides an additional imaging contrast that targets the short-length scales, i.e. intracellular structures This feature means that OGSE methods may provide extra contrast for detecting cancer or changes caused by treatment in diffusion-weighted MRI.

References:

(1) Beall et al., Science, 1976. (2) Callahan et al., Magn Reson Med, 1991. (3) Colvin et al., Proc Int Soc Magn Reson Med, 2006. (4) Xu et al., Magn Reson Med, 2009. (5) Parsons et al. Magn Reson Med 2006 (6) Galons et al. Magn Reson Med 2005 (7) Guo et al. J Magn Reson Imaging 2002