Characterization of lung tumor cell lines by amide proton transfer (APT) imaging in in-vitro system

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Introduction: Previous report shows that approximately 20% - 50% of lung nodules removed at surgery or by needle biopsy were benign (1), which clearly reveals that it is imperative to develop imaging methods yielding second-stage characterization test to distinguish benign from malignant cancer or differentiate cancer types or grades. Amide proton transfer (APT) imaging is one of the chemical exchange saturation transfer (CEST) imaging methods which images the exchange between protons of free tissue water and the amide groups (-NH) of endogenous mobile proteins and peptides (2). We previously showed that the method differentiated the two different lung cancer types in an orthotropic mouse model of lung cancer (3). The objectives of this study were to assess whether the in vitro APT-imaging is capable of distinguishing among cell types or between benign and malignant and whether the in vitro system can be a screening test for lung cancer cell lines. In this study, APT imaging was tested in three different cell lines, two malignant, adenocarinoma (A549) which is known as a radiation resistant and large cell neuroendocrine (H1299), and a benign (HSAEC1-KT) in vitro.

Materials and Methods: *Cell line preparation:* Lung tumor cell lines, A549 and H1299, were cultured in RPMI (Sigma) supplemented with 5% serum. Normal cells, HSAEC1, were cultured in serum free SAGM with supplements (Lonza). All cells were harvested by trypsin and followed by centrifugation. Cell pellets were re-suspended in PBS to a final concentration of 20 million cells per milliliter. 0.25 ml of the re-suspended cells was centrifuged in 200 µl PCR tubes for 15 seconds in a PCR centrifuge at a rate of 6000 rpm (VWR, Galaxy minister centrifuge). The supernatants were removed, additionally 250 µl of cells were added, and the centrifugation was repeated. MRI study was performed in the same tube after removing the supernatants and 1 hour after harvest. *MRI:* MRI was conducted in a 7T small animal MR scanner (Varian, Inc, Palo Alto, CA) with a 38 mm birdcage RF coil. APT imaging was performed on a 1 mm thick slice through all tumor cells simultaneously: gradient echo images were repeatedly obtained following each presaturation pulse (CW block pulse, B_1 = 2.3 µT, duration = 5 s) at 29 saturation points in steps of 0.5 ppm from 7 to -7 ppm. Other parameters were: TR/TE = 6.05/2.74 ms, FOV = 30 × 30 mm, flip angle = 20, matrix 128 × 64, NEX = 8. A control image with the presaturation pulse at an offset equal to 300 ppm was also acquired as a reference (S_0). Total acquisition time was approximately 20 minutes. *Image Analysis:* To obtain z-spectra, a region-of-interest, (ROI), was placed in the center of tube to avoid partial volume effects. The Z-spectra were fitted through all offsets on a pixel-by-pixel basis according to the procedure using the 12th-order polynomial fitting followed by the correction for B_0 inhomogeneity effect as Salhotra et al. reported (4). MTR (magnetization transfer ratio) asymmetry, MTR_{asym}, was defined as: $MTR_{asym} = S_{sat}(-\text{offset})/S_0 - S_{sat}(+\text{offset})/S_0$, where S_{sat} and S_0 are signal intensities on the images with presaturation pulse at 7 to -7 ppm and 300 ppm, respectively. The calculated MTR_{asym} map at a chosen offset (ppm) is called the APT-weighted image.

Results and Discussion: The analysis of APT imaging data shows a promising difference in APT signal among the cell lines. The corrected z-spectra are plotted in Figure 1. All cell lines exhibit asymmetric z-spectra. Each cell line demonstrated different level of MTR asymmetry plotted between 0 and 5 ppm where normal cells showed the lowest at any given frequency compared with the other two malignant cells, and a large cell neuroendocrine showed lower values from 1.5 ppm to 4 ppm than adenocarcinoma. Figures 3 shows an APT weighted image at 3 ppm superimposed on a T1 weighted image. The APT image at 3 ppm (Fig. 3) clearly shows a lower APT signal from the normal lung cell line (HSAEC1-KT), as compared to the malignant tumor lines (A549/H1299). This difference between cell lines is consistent between 1.5 ppm and 3.5 ppm offsets, as shown in Figure 2. The observed MR asymmetry appears shifter toward lower freq. CEST effect is a function of pH and temperature (5). In this study, we adjusted pH as 7.4 for all cell lines and measurement was conducted at magnetic room temperature 18°C, which shifts the peak MTR asymmetry. These lower temp and higher pH than those in vivo cancer would shift MTR asymmetry toward higher frequency while increased proton exchange rate in in vitro would shift MTR asymmetry to lower frequencies. Generally, CEST effect at 3.5 ppm is thought to be specific to the amide groups (-NH) of endogenous mobile proteins and peptides. Nevertheless, the data suggests that APT imaging can be used to delineate between different types of cells at 3.5 ppm, in which the difference between malignant cells and normal cells is enhanced.

The results showed that the in vitro APT-imaging is capable of distinguishing among cell types. This in vitro system could be applied to a large scale screening of a variety of cancer cell lines, aiding in delineating molecular heterogeneity in lung cancer cell lines. Further studies will include a comparison of the results between in vitro and in vivo orthotopic mouse model.

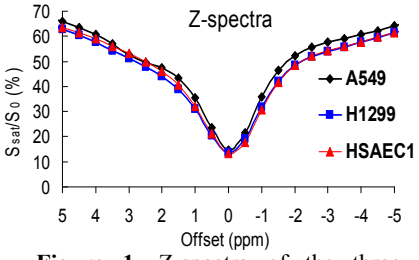

Figure 1. Z-spectra of the three tumor lines

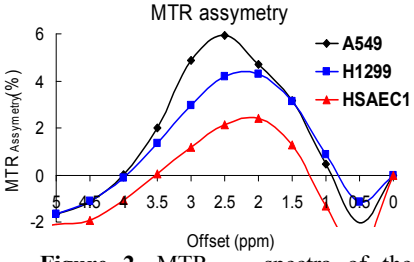

Figure 2. MTR_{assym} spectra of the three tumor cell lines.

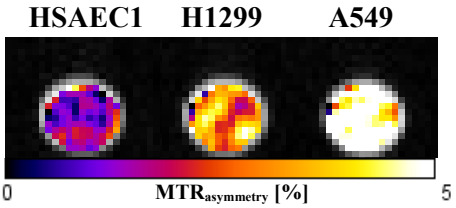

Figure 3. APT-weighted image at 3ppm superimposed on T1 weighted image for three different tumor cell lines.

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