Identification of Endogenous Proteins Correlated with Amide Proton Transfer (APT) Imaging Contrast using Proteomic Analysis

K. Yan[1], Z. Fu[2], J. Van Eyk[3], S. Wang[1], and J. Zhou[1]
[1]Radiology, Johns Hopkins University, Baltimore, MD, United States, [2]Pediatrics, Johns Hopkins University, Baltimore, MD, United States, [3]Bayview Proteomics Center, Johns Hopkins University, Baltimore, MD, United States

Introduction

Amide proton transfer (APT) imaging is a specific type of chemical exchange–dependent saturation transfer (CEST)[1,2] MRI technique in which amide protons of endogenous mobile proteins and peptides in tissue are detected.[3,4] When applied to imaging of brain tumors, the initial data suggest that APT could provide unique visual information about the presence and grade of brain tumors,[5] presumably due to increased cellular protein and peptide levels in glioma. Proteomics is defined as the large-scale characterization of the entire protein components of a cell, tissue or whole organism and can provide information about protein abundance, location, post-translational modification and protein-protein interactions in a proteome of a given biological system.[6] The aim of this study is to identify candidate cytosolic proteins that correlate with APT imaging of brain tumors by proteomic studies.

Materials and Methods

Five 9L glioma-bearing Fischer 344 rats were investigated in this study. APT images were acquired with a horizontal bore 4.7 T Biospec animal imager (Bruker Biospin, Billerica, MA) using a 32 mm × 32 mm field of view, 64 × 64 matrix size, and 2-mm interleaved slices. 2-mm-thickness coronal rat brain matrix and 3-mm-diameter tissue biopsy punch were used in tissue sample preparation. Total proteins from tumor and contralateral normal tissues were extracted and subjected to 2-D fluorescence difference gel electrophoresis (2-D DIGE) analysis. Mass spectrometry (MALDI-TOF-TOF and LTQ-Orbitrap) was performed to identify proteins.

Results and discussion

T2 and APT images acquired on the 9L gliosarcoma tumor model in a Fisher 344 rat (postimplantation day 12) were shown in Figure 1. The tumor showed hyperintensity, compared to contralateral brain tissue, on both the T2 and APT images, but the lesion identified by APT was much cleaner, corresponding well to H&E histology. The average APT signals were higher in tumor than in contralateral (1.88 ± 0.45% *vs.* -1.74 ± 0.38%; $p < 0.001$). For proteomic studies, tissue samples with same volumes from tumor and contralateral normal regions (Figure 2) were prepared. The protein expression profiles of tumor and normal tissues (Figure 3) showed remarkable qualitative and quantitative proteomic pattern differences. Over 1000 protein spots were detected on 2-DE gels. The 72 most abundant protein spots were picked up for MS and 45 proteins were identified successfully. Particularly, six cytosolic proteins, including annexin A5 (ANXA5), translationally-controlled tumor protein (TPT1), cellular retinoic acid-binding protein 1 (CRABP1), thioredoxin (TXN), protein S100-A4 (S100A4) and protein S100-A6 (S100A6), showed significant up-regulated expressions in tumor. These proteins can be assumed to cause the APT hyperintensity in the tumor.

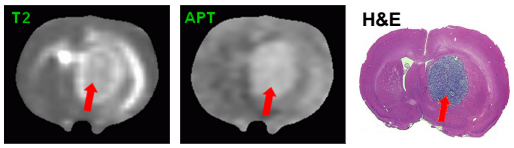

Figure 1. MR images and histology for the 9L gliosarcoma tumor model (red arrow) in a Fisher 344 rat (12 days after implantation)

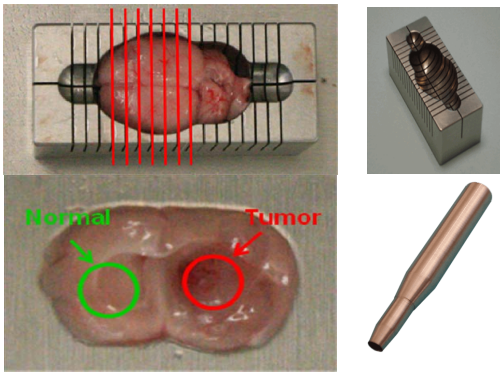

Figure 2. Tissue samples from tumor and contralateral normal regions with same volumes (2-mm-thick and 3-mm-diameter)

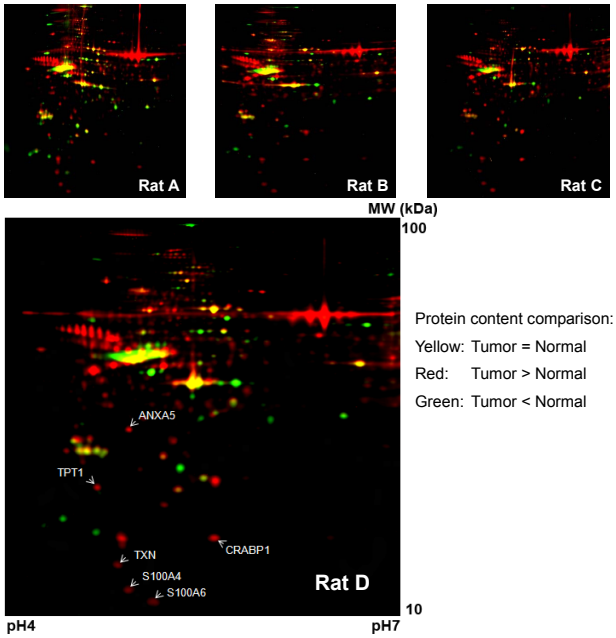

Figure 3. Combined 2-D DIGE gel maps of proteins in tumor (Cy5 labeled) and normal (Cy3 labeled) tissues

Conclusions

The proteomic results demonstrate six up-regulated cytosolic proteins within tumor tissue (compared to contralateral brain tissue) that may be correlated with the hyperintense APT imaging signal in the tumor region.

References: (1) Ward et al. *JMR* 2000, 143, 79. (2) Zhang et al. *JACS* 2001, 123, 1517. (3) Zhou et al. *MRM* 2003, 50, 1120. (4) Sun et al. *JCBFM* 2007, 27, 1129. (5) Wen et al. *NeuroImage* 2010, 51, 66. (6) Wilkins et al. *Biot. Genet. Eng. Rev.* 1996, 13, 19.