Magnetic Resonance Image-Guided Proteomic Analysis of Human Glioblastoma Multiforme: A Two-Dimensional Gel Electrophoresis Study

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Introduction

Proteomic expression profiles are widely used to correlate with clinical presentation patterns, surrogate markers of disease, and pathological evaluations to identify new cancer protein markers. Magnetic resonance imaging is an important diagnostic tool that plays an important role in the clinical management of patients. MRI can provide high spatial resolution of molecular signatures of normal and diseased tissue such as in solid tumors. Glioblastoma multiforme (GBM) is the most common primary and malignant brain tumor in human with the overall 5-yr survival rate of less than 6% (1). Previously we have shown that peptide expression profiles obtained from mass spectroscopy analysis correlate to gadolinium contrast enhancement patterns on T1-weighted magnetic resonance (MR) images in human GBM (2). We concluded that the contrast enhancing regions of the tumors exhibit a high degree of low molecular weight peptides due potentially to proteolysis and remodeling in these regions of the tumor as compared to the non-enhancing areas. The purpose of this study is to investigate spatial changes in high molecular weight protein expression profiles related to contrast enhancement of gadolinium-DTPA using two-dimensional gel electrophoresis and to identify those differentially expressed proteins that may serve as potential tumor markers and targets for further evaluation.

Materials and Methods

Patients with MR images and confirmed diagnosis of GBM were enrolled in the study. Images were acquired with a1.5-T MRI scanner (GE Medical Systems, Milwaukee, WI) using 28 cm x 28 cm field of view, 256 x 256 matrix size, and 2-mm interleaved slices. Pre-contrast spin echo T1-weighted and fast spin echo T2-weighted images were obtained. After the administration of a single dose (0.1 mmol/kg) gadopentate dimeglumine (Magnevist, Berlex Laboratories, Wayne, NJ), spin echo T1-weighted images were obtained. Sites for tissue sampling were determined on the basis of the post-contrast T1-weighted images. Tissue biopsies were obtained under the guidance of T1-weighted MRI and solubilized in lysis buffer (8M urea, 10% CHAPS, with protease inhibitor cocktail (Roche, Germany)). Lysed tissue samples were centrifuged and supernatants were removed and stored at -80 ^{o}C. We examined the protein expression profiles using conventional two-dimensional gel electrophoresis (2D-GE) and two-dimensional differential gel electrophoresis (2D-DIGE) from contrast-enhancing (CE) and non-enhancing (NE) regions within a single tumor mass in four different patients. For 2D-DIGE analysis, proteins samples from CE and NE regions within a given tumor were labeled with Cy3 and Cy5 fluorescent dyes respectively according to manufacture's protocol (Amersham Biosciences, Uppsala, Sweden). The labeled samples were mixed and followed by 2D gel electrophoresis. Gel images were obtained using Typhoon 9400 laser scanner (Amersham Biosciences) and analyzed using ImageQuant and DeCyder (Amersham Biosciences) image analysis software.

Results

Protein profiles from CE and NE regions within a given tumor have different proteomic patterns suggesting an altered gene expression profile that correlates with detectable tissue imaging parameters (Figure 1). Comparing protein expression profiles generated by conventional 2D-GE with that of 2D-DIGE, we found 2D-DIGE reveals significant differences in protein expression between CE and NE regions indicating improved sensitivity of the latter technology. We also found distinct differences in protein expression profiles among different patient despite similar imaging and histological features suggesting tissue heterogeneity within solid tumors. This difference arises from the contrast enhancing regions, where each patient profile is unique amongst the 1000s proteins visualized using 2D-DIGE. These differences occur despite no discernable differences using CE-MRI and histological analysis using H&E. Detailed analysis and comparison of expression profiles among all the patients revealed only three proteins similarly regulated across all four patients patients. One of these possible markers was transthyretin after sequencing protein obtained from the gels using standard procedures.

Discussion

To test our hypothesis that MR contrast enhancement signatures of disease will correlate with altered protein patterns, we compared the proteomic fingerprint of tissue samples obtained from four GBM patients based on differences in contrast enhancement. Results from this investigation clearly show the existence of protein profile differences that correlate to regions of MR contrast enhancement. The extreme differences observed between the CE regions of the tumor between patients implies that each individual may have unique protein patterns for their tumor which implies that for GBM individualized therapies may need to be developed. However, heterogeneous cell population and tissue type within regions of similar imagining properties may complicate the protein profile analysis, it may therefore be necessary to isolate particular cell types for further analysis. Finally, molecular imaging reagents with higher specificity that can target protein specific tissue markers may need to also be developed to validate these differences.

Conclusion

These results demonstrate that major differences in protein expression patterns within a tumor can be correlated to radiographic findings. Regions of contrast enhancement in an individuals tumor show different high molecular weight protein profile that the non-enhancing region of the tumor. Interestingly, the protein profiles from the CE regions of the tumor seem to be unique for each individual implying that the tumors all expressing unique signatures. This conclusion implies that GBM may require individualized therapies where molecular imaging can guide which treatments are valid noninvasively. We conclude that contrast enhanced MRI can serve as a powerful tool for characterizing different regions of tumors prior to proteomic analysis using 2D-GE and 2D-DIGE and 2D-DIGE is an excellent technique for comparing image-guided biopsies within regions of an individual patients tumor.

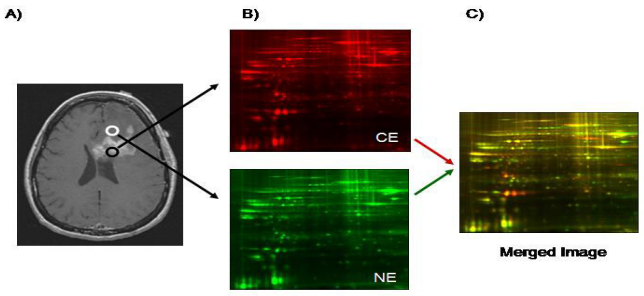

Figure 1. Representative correlation between post-contrast T1-weighted MR images and 2D-DIGE protein profiles. **(A)** Postcontrast T1-weighted MR image with the sites of tissue selection indicated by the circles (Black circle = CE region, White circle = NE region). **(B)** The corresponding 2D-DIGE protein expression profile of the CE (Cy3-labeled, red) and NE (Cy5-labeled, green) regions. (C) Merged image of both CE and NE regions. Yellow spots indicated proteins with similar expression level. Red spots indicate proteins overexpressed in the CE region and green spots indicate proteins overexpressed in the NE region.

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