# Identification of serum protein profiles of glioblastoma multiforme patients: using image-guided microarray analysis of tumor samples to identify serum markers

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**Introduction:**

Glioblastoma multiforme (GBM) is a primary brain tumor with poor prognosis and low survival rate.[1-2] Like other solid tumors, GBM is heterogeneous in morphology. Thus tissue sampling for microarray analysis from these tumors can give rise to varying results depending on the location of biopsy. CE-MRI using Gd(DTPA) has been demonstrated to be a powerful technique to identify regions with increased vascular permeability and vessel density.[3] We have proposed that the use of contrast-enhanced MRI (CE-MRI) to guide tissue sampling for microarray analysis can improve target identification in these solid tumors. In addition, targets identified in the CE regions (where the blood-brain-barrier is disrupted) may provide a panel of serum markers for identification and monitoring disease progression.

**Materials and Methods:**

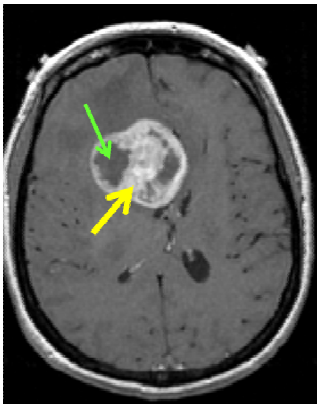
Patients diagnosed with GBM, without any prior surgical, chemotherapy or radiotherapy procedures were scanned on a GE 1.5T MRI scanner using standard T1- and T2-weighted pulse sequences (TR/TE 500/30, TR/TE 2500/90, ET 8 respectively, 256x256, FOV 28cm x 28cm, NEX1, 2mm interleaved slicing ) and Magnevist (Gd(DTPA), Berlex Inc., NJ, 0.1 mmol/kg) as contrast agent. Samples from regions that have contrast agent accumulation (contrast-enhancing, CE) and regions that do not take up contrast agent (non-enhancing, NE) were collected for gene expression profiling using oligonucleotide microarray analysis (Figure 1). Patient serum samples were also collected for protein expression quantification using ELISA assay. Tissue and serum samples from healthy individuals were used as controls.

**Results:**

Tissue samples from the CE and NE regions of 13 patients reveal significantly distinct gene expression patterns (Table 1). These results show insulin-like growth factor binding protein -2 (IGFBP-2), IGFBP-3, IGFBP-5, acidic FGF (aFGF), heat shock protein (HSP) 90 and autotaxin were all up-regulated in the CE regions as compared to the NE region. Immunohistochemical staining confirmed correlation of protein expression patterns with the observed genomic profile. Since in the CE region of the tumor the BBB is compromised, we evaluated the presence of proteins with low molecular weight (MW<30 kD) in the serum. We hypothesized that these proteins could enter systemic circulation and be detected in the patient serum due to the high vascular permeability. Preliminary results from ELISA performed on 7 patients indicate that IGFBP-2 has a higher mean value ($86.1 \pm 29.1$ ng/ml) in GBM serum as compared to healthy individuals ($55.7 \pm 9.9$ ng/ml). However, other potential markers such as IGFBP-3 and aFGF do not exhibit any difference between GBM patients and controls.

**Conclusion:**

We have found that CE-MRI can serve as a powerful tool for characterizing different regions of heterogeneous solid tumors for microarray analysis. CE-MRI using the clinical MRI agent Gd(DTPA) can reveal imaging features associated with increased vascular permeability and vessel density, and areas of fluid accumulation and necrosis. We have observed that differences in spatial resolution in the tumor correlate to changes in gene expression profiles. We conclude CE-MRI guided sampling and microarray analysis can be used to evaluate targets in permeable regions of the tumor. These targets can be further screened to identify serum profiles for diagnostic and clinical monitoring of the patient before and after therapeutic intervention.



**References:**

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2. Knopp M, Weiss E, Sinn H, et al. Pathophysiologic basis of contrast enhancement in breast tumors. Journal of Magnetic Resonance Imaging 1999; 10:260-266.
3. Tynninen O, Aronen H, Ruhala M, et al. MRI enhancement and microvascular density in gliomas. Investigative Radiology 1999; 34:427-434.

**Figure 1.** T-1 weighted CE-MRI of representative patient with GBM. The thick (yellow) and thin (green) arrows indicate the contrast-enhanced (CE) and non-enhanced (NE) regions, respectively.

**Table 1.** Average gene expression profiles from GBM and healthy individuals. The table shows the gene name, their accession numbers, average expression level intensity for the contrast-enhanced (CE), non-enhanced (NE) regions of 13 GBM patients, and the normal brain tissue of 5 healthy individuals.

| Gene | Acc# | Avg Exp Level, CE Region (n=13) | Avg Exp Level, NE Region (n=13) | Avg Exp Level, Normal Brain (n=5) |
|---|---|---|---|---|
| **IGFBP-2** | X16302 | 12582 ± 5121 | 9542 ± 6770 | 1519 ± 1132 |
| **IGFBP-3** | M35878 | 19525 ± 8466 | 12389 ± 9014 | 1458 ± 1319 |
| **IGFBP-5** | L27560 | 23576 ± 8923 | 18210 ± 12299 | 2829 ± 3119 |
| **acidic FGF** | AF010187 | 4530 ± 933 | 3683 ± 602 | 2303 ± 1213 |
| **autotaxin** | L35594 | 16480 ± 7567 | 13213 ± 8940 | 102870 ± 39164 |
| **HSP 90, alpha unit** | X15183 | 38192 ± 8032 | 33291 ± 11112 | 63746 ± 22042 |
| **HSP 90, beta unit** | M16660 | 29929 ± 4212 | 17742 ± 7061 | 13657 ± 4821 |