JPP JOURNAL OF PROTEINS AND PROTEOMICS
5(1), January-April 2014, pp. 55-64

Research Article

ALTERED EXPRESSION OF ISOCITRATE DEHYDROGENASE1 IN ASTROCYTOMA (III AND IV) AND OLIGODENDROGLIOMA (III) BRAIN TUMORS

Mehdi Pooladi[1], SolmazKhaghaniRazi Abad[1], Niloofar Nazarian[1], Leila Firouzi-Dalvand[2] and Mansooreh Hooshiyar[1]

[1]*Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.*

[2]*Department of Microbiology, North Tehran Branch, Islamic Azad University, Tehran, Iran.*

Abstract: Gliomas are the most common type of primary brain tumors. Isocitrate dehydrogenases (IDH) catalyze the oxidative decarboxylation of isocitrate to a-ketoglutrarate and regulation of the enzymatic activity of IDHs is crucial for their biological functions. The causal relationship between IDH mutations and glioma genesis is only partly understood. We extracted proteins of tumor and normal brain tissues, quantitated them by Bradford's method and evaluated the differences. We separated proteins in the two sets by two-dimensional gel (2DG) electrophoresis and the resulting spots were then analyzed and compared using statistical data and Progenesis SameSpots software. Spots were identified by isoelectric pH, molecular weights and data banks and validated using mass spectrometric identification. The 2D gels showed 800 total spots for astrocytoma (III), 876 total spots for astrocytoma (IV) and total of 1328 spots for oligodendroglioma (III). Among them the statistically significant protein spots (P<0.05) indicated IDH_1 protein to be up-regulated. Proteomics analysis revealed an unexpected alteration in the expression of certain protein networks in IDH mutation gliomas. IDH_1 is located in the cytosol and play distinctive roles in cellular metabolism**.** IDH_1 is a metabolic enzyme implicated in cancer. Thus, IDH_1 might be a candidate biomarker in glioma tumors**,**

Keywords: asterocytoma; oligodendroglioma; proteomics; IDH_1 and 2DG Electrophoresis

Introduction

Glioma is the most common type of primary brain tumors, and is grouped into four grades according to the WHO criteria (Yan *et al.*, 2009; Sonoda and Tominaga, 2010). Gliomas are a classification of nervous system tumors arising from glial cells, the most common of which are astrocyromas and oligodendrogliomas, arising from astrocytes and oligodendroglial, respectively (Borodovsky *et al.*, 2013). Isocitrate dehydrogenases (IDH) catalyze the oxidative decarboxylation of isocitrate to α-ketoglutrarate, and regulation of the enzymatic activity of IDHs is crucial for their biological functions (Xu *et al.*, 2004). Mutations in IDH seem to play an important role in the formation of specific subtypes of gliomas (Sonoda and Tominaga, 2010). Point mutations in the IDH enzymes, IDH_1 and IDH_2, are found in the majority of WHO grade II and III diffuse gliomas - both astrocytic and oligodendroglial - as well as secondary glioblastoma multiforme (GBMs), but they are almost entirely absent from primary GBM (Huse *et al.*, 2010; Capper *et al.*, 2010; Yan *et al.*, 2009).

The most common IDH_1 mutations in glioma (>95%) result in an amino acid substitution at $arginine_{132}$ (R132), which resides in the enzyme's

Corresponding Author: **Mehdi Pooladi**
E-mail: Mehdi.pooladi7@gmail.com

Received: January 25, 2014
Accepted: April 26, 2014
Published: April 30, 2014

active site (Turcan *et al*., 2012). IDH_2 mutations were highly conserved, confined to a single residue, R172, respectively, and most frequently a single allele was mutated with the wild type allele retained (Parsons *et al*., 2008). IDH_1 is localized to the cytoplasm and peroxisomes. It is highly expressed in the liver and to a lesser level in other tissues, but, IDH_2 is localized to the mitochondria, is highly expressed in heart, muscle, and activated lymphocytes and moderately in other tissues (Krell *et al*., 2011; Reitman and Yan, 2010).

The causal relationship between IDH mutations and glioma genesis is only partly understood. The mutational profile of IDH1 and IDH2 is peculiar as the mutations affect only single evolutionarily conserved residues (arginine R132 and R172, respectively). The arginines are localized in the substrate binding site of the isozymes, were hydrophilic interactions between the arginine and both α and β carboxylate of isocitrate are formed (Bleeker *et al*., 2011; Atai *et al*., 2011; Zhang *et al*., 2013).

In the present study, we investigated the IDH_1 expression change in human brain astrocytoma (III and IV) and oligodendroglioma (III) tumors. To get an understanding of data and specific software for molecular diagnosis of glioma, we extracted proteins of tumoral and normal brain tissues and evaluated the protein quantities. We separated proteins by two-dimensional gel electrophoresis and identified alternation in spot characteristics using statistical data, specific software (Progenesis Same Spots) and MALDI TOF-TOF.

Materials and Methods

Patient samples

Tissues were obtained, with informed consent and institutional review board approval, from patients undergoing tumor resectioning. For this study, all individuals filled a written informed consent form. Astrocytoma (III and IV) and oligodendroglial (III) tumors were surgically removed at Shohada Tajrish Hospital. The tumors were classified by a team of neuropathologists according to the guidelines of the World Health Organization (WHO) classification of tumors of the central nervous system. Ten tumors [4 astrocytoma (III), 3 astrocytoma (IV), and 3 oligodendroglioma (III)] from surgery operated patients with malignant glioma have been separated. Non-tumoral brain tissues were obtained from normal areas (either grey or white matter) of brain tissues removed from patient undergoing non-tumor epileptic surgery. In all phases of research, ethical issues have been considered. Also, informed consent from patients or their relatives to participate in this research were taken.

Tissue and sample preparation

Tissue samples of both tumoral and normal brain tissue were snap-frozen immediately after operation in liquid nitrogen and stored at -80°C until used for proteomic analysis. To obtain tissue extracts, the samples were fragmented into suitable pieces and were homogenized on ice in lysis buffer II consisting of lysis buffer I [7M urea, 2M thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 0.2% 100×Bio-Lyte 3/10}, dithiothreitol (DTT)] and 1 mM ampholyte and protease inhibitor. Cell lysis was completed by subsequent sonication (4×30 pulses). The samples were then centrifuged at 20,000 g at 4°C for 30 minutes to remove insoluble debris. The supernatants were combined with 100% acetone and centrifuged at 15,000 g, and then the supernatants were decanted and removed (3 times). Acetone (100%) was added to the protein precipitant and kept at -20°C overnight. The samples were then centrifuged again at 15,000 g and the precipitant incubated for 1 hour at room temperature. The protein samples were dissolved in rehydration buffer [8 M urea, 1% CHAPS, DTT, ampholyte pH (4) and protease inhibitor]. Protein concentrations were determined using the standard Bradford assay, and the protein extracts were then separated and used for 2D gel electrophoresis.

Two-dimensional gel electrophoresis

The isoelectric focusing for first-dimensional electrophoresis was performed using 18 cm, pH 3–10 immobilized pH gradient (IPG) strips. The samples were diluted in a solution containing rehydration buffer, IPG buffer, and DTT to reach

a final protein amount of 500 µg per strip. The strips were subsequently subjected to voltage gradient as described in the instructions of the manufacturer. Once focused, the IPG strips were equilibrated twice for 15 minutes in equilibration buffer I [50 mM Tris-Hcl (pH: 8.8), 6 M urea, 30% glycerol, 2% sodium dodecyl sulfate (SDS) and DTT] and equilibration buffer II [1 mL lysis buffer I, ampholyte pH (4) and protease inhibitor]. The second-dimension SDS-PAGE was carried out using 12% polyacrylamide gel. Following SDS-PAGE, the gels were stained using the Coomassie Brilliant Blue method (overnight).

Image analysis

Analytical gels were scanned by Densitometer GS-800 (BioRad) scanner at 600 dpi in tagged image file format (TIFF). Image Master™ 2D platinum v6.0 software was then used to extract and digitize data from graphical images of scanned gels through detecting, normalizing, matching and comparing protein spots according to their volume percent. The gel images were analyzed automatically as well as by Progenesis SameSpots software to identify spots differentially expressed between astrocytoma tumors (III,IV) and control samples, oligodendroglial tumor (III) and normal tissue and astrocytoma (III) and astrocytoma (IV). The spots were carefully matched individually and only spots that showed a definite difference were defined as altered. Spots were detected by isoelectric pH, molecular weights, databanks and comparison with previous research.

Mass spectrometric (MS) analysis

Spots were manually excised from 2-DE gels. Each gel plug was soaked in 100 µL of washing solution (50% methanol, 50mM NH_4HCO_3) to re-swell and was then washed two more times in the same solution. The gel plugs were further washed twice in 75% (v/v) acetonitrile (ACN), before being completely dried. Samples were then re-hydrated by adding freshly prepared trypsin solution (0.5 µg modified porcin trypsin in 25ìL 20 mM NH_4HCO_3), and were incubated for 240 min at 37°C. Peptides were extracted from the gel plugs by washing twice in 100 µL of 50% ACN, 0.1% TFA and transferred in solution to a fresh 96 well plate, where samples were dried. Tryptic peptides were resuspended in 3 µL of 50% ACN, 0.1% TFA. Subsequently, 0.3 µL of resuspended suspension was mixed (while wet) with 0.3 µL of a 90% saturated µ-cyano-4-hydroxycinnamic acid (CHCA) in 50% ACN, 0.1% TFA. The identity of differentially expressed proteins ($P < 0.05$ and fold >2) was established using MALDI TOF TOF MS (AB Sciex 5800).

Results and Discussion

Using 2D-gel proteomic analysis, we compared protein expression patterns between astrocytoma (grades III and IV) and oligodendroglioma (grade III) samples relative to control tissue (Figure 1). The 2D-gel electrophoresis revealed consistent protein profiles for each group. Simple statistical test were used to establish a putative hierarchy in which the change in protein level were ranked according to a cutoff point with $P<0.05$. The 2D gel showed total of 800 spots for astrocytoma (III). A total of 343 spots showed statistically significant differences (student's *t*-test; $P<0.05$) in gel, of which 164 spots exhibited up-regulation in expression level, whereas the remaining 179 spots were down-regulated in astrocytoma tumor relative to normal tissue. Amongst the statistically significant protein spots ($p<0.05$), IDH_1 proteins (first spot: as shown in Figures 2 and 3) were definitely present with isoelectric pH 6.41 and molecular weight 46 kDa, detected with an up-regulation of about 2 (fold=2) (Figure 4). In addition, a total of 876 protein spots were observed for astrocytoma (IV). A total of 420 spots showed statistically significant differences (student's *t*-test; $p<0.05$) in gel, of which 188 spots exhibited up-regulation in expression level, whereas the remaining 232 spots were down-regulated in astrocytoma tumor relative to normal tissue. Amongst the statistically significant protein spots ($p<0.05$), IDH_1 proteins were definitely present (first spot: as shown in Figures 2 and 3) with isoelectric pH 6.66 and molecular weight 49 kDa, detected with an up-regulation of about 1.8 (fold=1.8) (Figure 5).

Amongst the total 1328 spots observed for oligodendroglioma (III), a total of 433 spots showed statistically significant differences (student's *t*-test; $p<0.05$) in gel, of which 157 spots

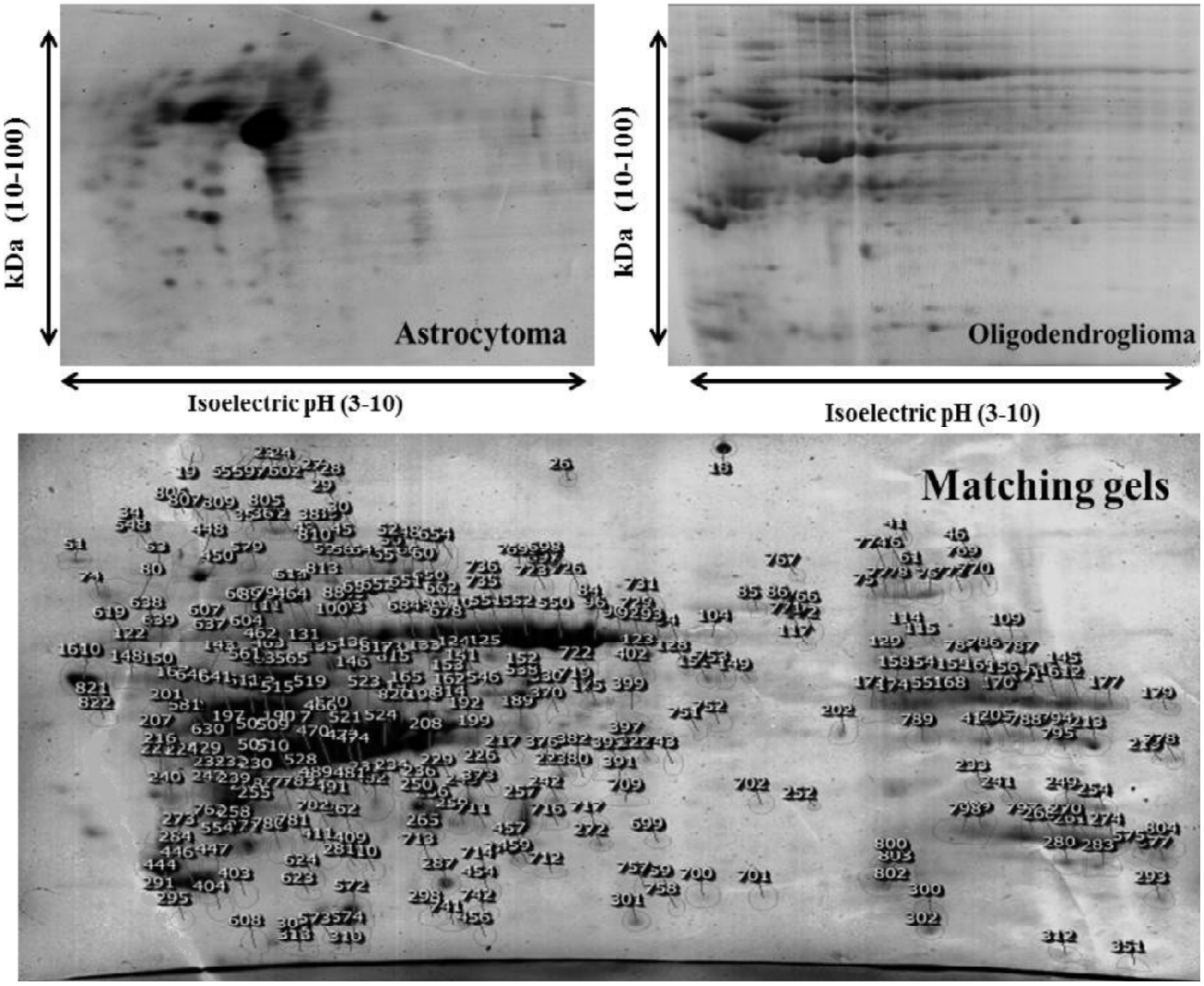

Figure 1: **Two-dimensional gel electrophoresis images of oligodendroglioma and astrocytoma tumors. Proteins were separated on the basis of isoelectric pH (3-10) and molecular weight (10-100) and the gels were aligned for comparison.**

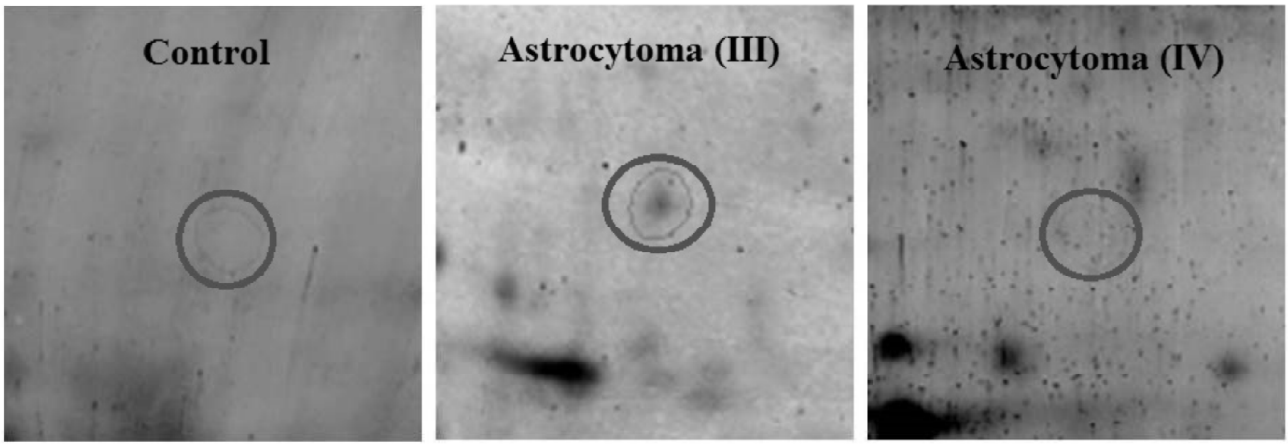

Figure 2: **Comparison of levels of expression changes. Spots (IDH_1) in astrocytoma (III) and astrocytoma (IV) brain tumors relative to normal brain tissue are shown.**

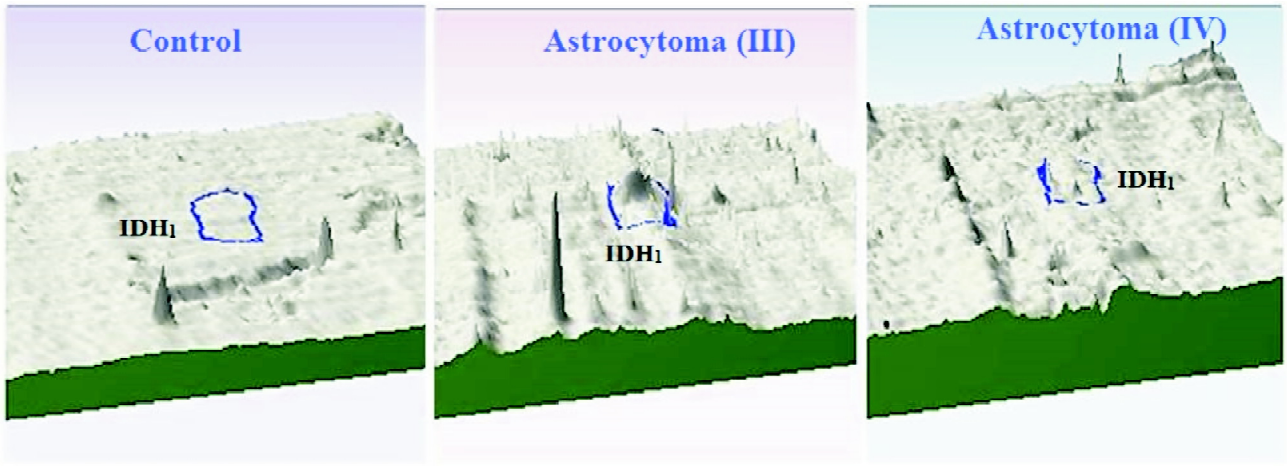

Figure 3: **3D images of IDH_1 protein in tissue of astrocytoma (III and IV) tumors.**

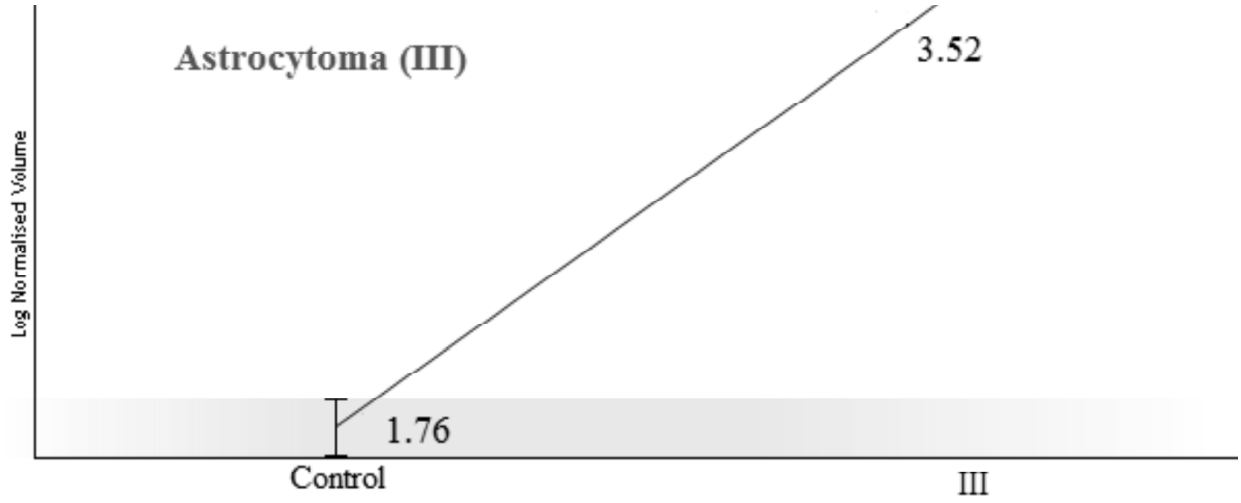

Figure 4: **IDH_1 protein showed an up-regulation of about 2 (fold=2) in astrocytoma (III) brain tumors compared to normal brain tissue**

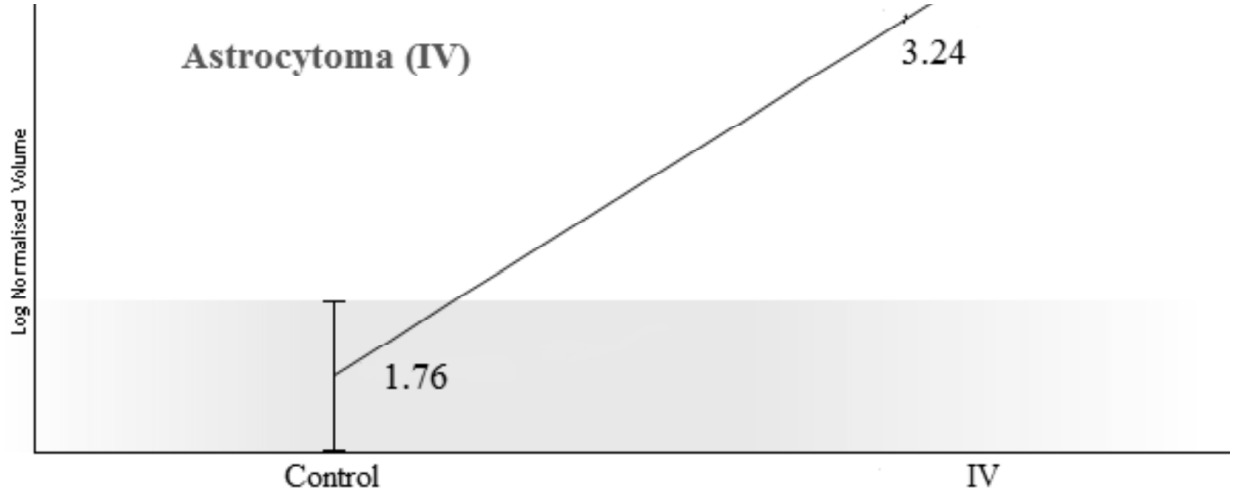

Figure 5: **IDH_1 protein showed an up-regulation of about 1.8 (fold=1.8) in astrocytoma (IV) brain tumors compared to normal brain tissue**

exhibited up-regulation in expression level, whereas the remaining 276 spots were down-regulated in astrocytoma tumor relative to normal tissue. Amongst the statistically significant protein spots (P<0.05), IDH_1 proteins were also definitely present (first spot: as shown in Figure 6) with isoelectric pH 6.77 and molecular weight 48 kDa, detected with an up-regulation of about 2.8 (fold=2.8) (Figure 7). As can be seen in the figure, oligodendroglioma show higher expression than astrocytoma changes, and also grade III astrocytoma showed more expression than grade IV astrocytoma changes.

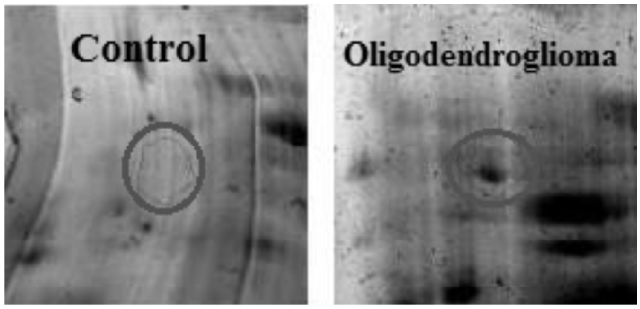

Figure 6: **Comparison of level of expression changes. Spots (IDH_1) in oligodendroglioma (III) brain tumors relative to normal brain tissue.**

For further understanding of rates of change, each of the tumors (oligodendroglioma III, astrocytoma III, and astrocytoma IV) was compared with the controls. Molecular weight and isoelectric pH values are displayed in Table 1 and the trend lines are shown in Figure 8. In addition, statistical analyses were examined closely and presented in Table 2.

Protein identification as above was validated by MALDI TOF TOF analysis (Matrix Science, Mascot search results). Moreover, we checked IDH_1 expression (up-regulated) in oligodendroglioma (III), astrocytoma (III) and astrocytoma (IV) tumors relative to control

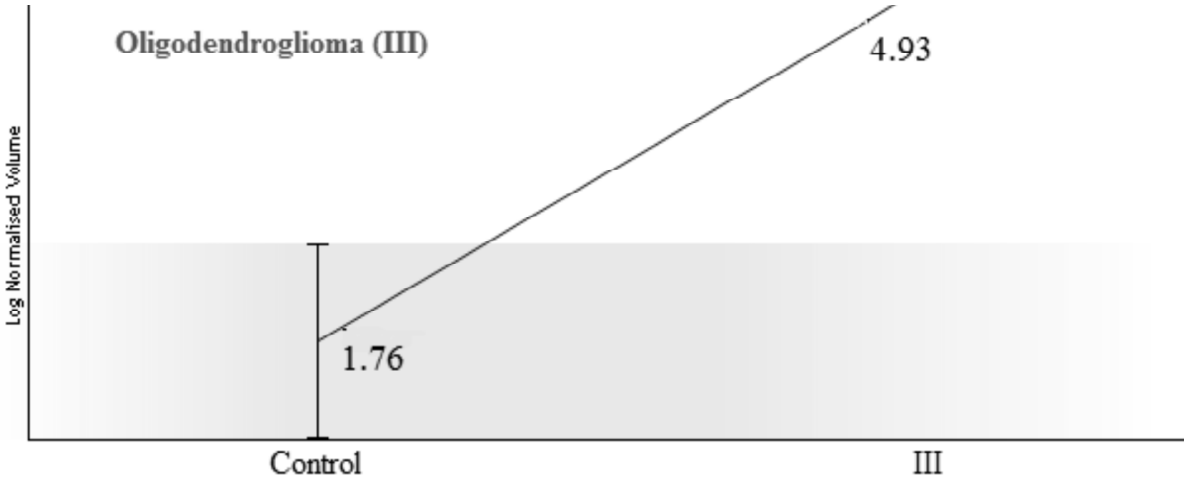

Figure 7: **IDH_1 protein showed an up-regulation of about 2.8 (fold=2.8) in oligodendroglioma (III) brain tumors relative to normal brain tissue.**

Table 1
Molecular weight and isoelectric pH of IDH_1 of each of the tumors (oligodendroglioma III, astrocytoma III and astrocytoma IV) compared to control independently.

	tumor	*grade*	*sex*	*age*	*IDH*		
					P<0.05	*PI*	*MW*
Case 1	oligodendroglioma	**III**	**Man**	**48**	1.549e-013	**6.67**	**47**
Case 2	oligodendroglioma	**III**	**Man**	**69**	1.876e-008	**6.75**	**48**
Case 3	oligodendroglioma	**III**	**Woman**	**63**	3.533e-008	**7**	**47**
Case 4	astrocytoma	**III**	**Man**	**39**	1.731e-006	**6.1**	**49**
Case 5	astrocytoma	**III**	**Man**	**60**	2.804e-006	**6.75**	**45**
Case 6	astrocytoma	**III**	**Woman**	**51**	1.240e-005	**6.76**	**47**
Case 7	astrocytoma	**III**	**Man**	**62**	3.443e-006	**6.43**	**48**
Case 8	astrocytoma	**IV**	**Woman**	**49**	1.311e-005	**6.61**	**51**
Case 9	astrocytoma	**IV**	**Man**	**55**	1.048e-007	**6.68**	**47**
Case 10	astrocytoma	**IV**	**Man**	**56**	4.839e-008	**6.52**	**48**

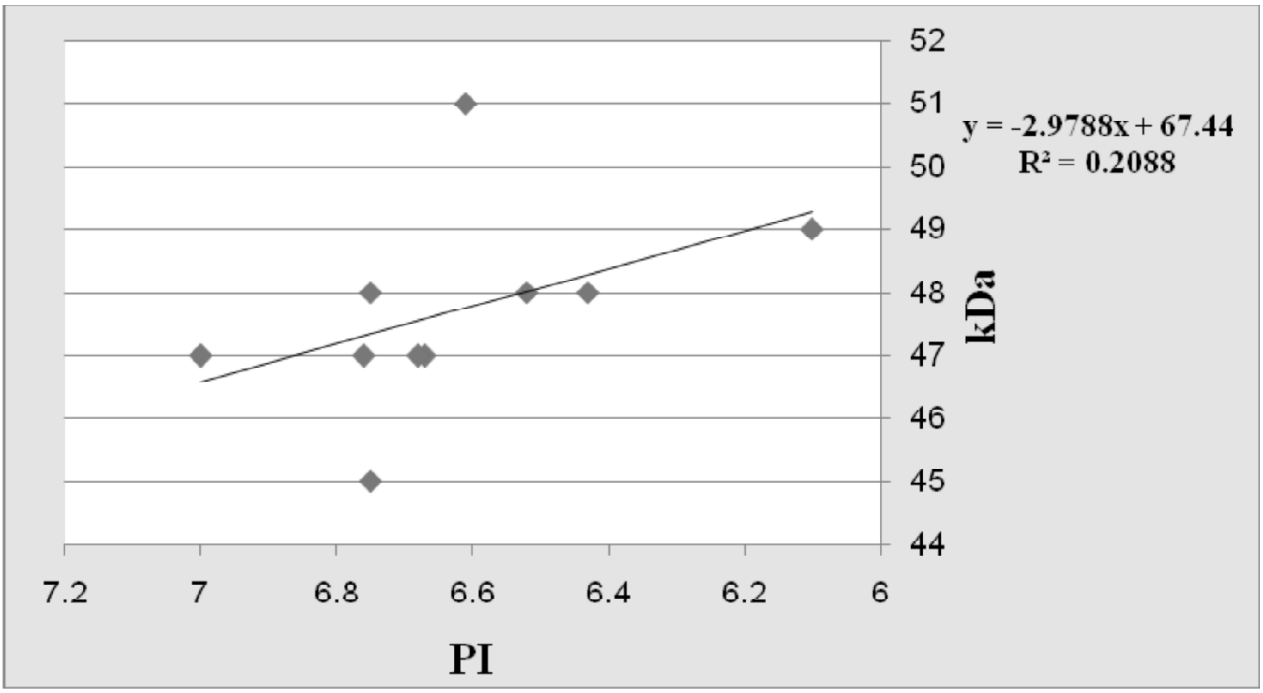

Figure 8: **Diagram of scatter and trendline drawings for the molecular weight and isoelectric pH, based on data from table 1.**

identified by MALDI TOF TOF. Levels of IDH_1 spots were markedly higher in tumor than non-tumor. The data from MALDI TOF TOF analysis are shown in Table 3.

Proteomics analysis revealed an unexpected alteration in the expression of certain protein networks in IDH mutation gliomas. The energy protein malnutrition (EPM) proteins, which crosslink actin, the cytoskeleton, and the plasma membrane is one of such network (Thirant *et al.*, 2011). Knowledge about the molecular biology of cancer, in clouding CNS tumours, continues to increase. Having a dynamic classification of tumors enables the integration of newly discovered markers to help determine prognosis and likelihood of therapeutic response (Jansen *et al.*, 2010). Recently, molecular markers have been increasingly used for the assessment and management of malignant glioma. Some molecular signatures are used diagnostically to help pathologists classify tumors (Huse *et al.*, 2010).

Table 2
Statistical analysis of molecular weight and isoelectric pH for IDH_1

	Valid	*Missing*	*Mean*	*Median*	*Error of Mean*	*Variance*	*Min*	*Max*	*Rande*
PI	10	0	6.63	6.61	0.057	0.003	6.1	7	0.9
MW	10	0	47.7	47.5	2.456	6.032	45	51	7

Table 3
MALDI TOF TOF analysis of IDH_1 protein matching the same set of peptides in databank (NCBI).

	Expressed Proteins Change	*Fold Change*	*Number of Peptides*	*Score*	*Matches*	*Sequences Coverage*
Oligodendroglioma (III)	Up-Regulated	2.8	20	202	57(61)	61
Astrocytoma (III)	Up-Regulated	2	18	137	39(44)	44
Astrocytoma (IV)	Up-Regulated	1.8	17	137	36(37)	37

IDH_1 is located in the cytosol and the peroxisome and produces NADPH (Marie and Shinjo, 2011). IDH_1 and IDH_2 play distinctive roles in cellular metabolism, with IDH_1 involved in lipid metabolism (in peroxisome NADPH contributes to cholesterol synthesis) and glucose sensing and IDH2 involved in the regulation of oxidative respiration (Takahashi *et al.*, 2013; Reitman *et al.*, 2010). This was a major surprise, because IDH_1, a metabolic enzyme, had never before been implicated in cancer (Garber 2010; Dang *et al.*, 2009). Normally, the IDH enzymes catalyze the conversion of isocitrate to á ketoglutarate and NAP^+ to NADPH, both metabolites ultimately derived from glucose in the course of generating energy for the cell and α-ketoglutarate is also required for the activity of about 60 enzymes known as dioxygenases (Marie and Shinjo, 2011; Thirant *et al.*, 2011). Although IDH_1 play a crucial role in the defense against oxidative stress, they are inactivated by oxidation. Lipid peroxidation products, singlet oxygen, hypochlorous acid, ROS, nitric oxide, and peroxynitrite all inactivate the enzymes. This process is likely mediated by modifications including glutathionylation in the presence of high levels of oxidized glutathione (Reitman and Yan, 2010). Gliomas with IDH mutations were clinically and genetically distinct from gliomas with wild-type IDH genes. Notably, two subtypes of gliomas of WHO grade II or III (astrocytoma and oligodendrogliomas) often carried IDH mutations but no other genetic alterations that are detectable relatively early during the progression of gliomas. Mutations in IDH1or IDH_2 were not identified in any pilocytic astrocytoma of WHO grade I, indicating that these tumors arise through a different mechanism (Garrett-Bakelmanand Melnick, 2013; Kim and Liau, 2012). Several studies have investigated the utility of IDH mutations as diagnostic marker and our study also confirms the same. Combination of IDH_1 genetic status can serve as specific marker to differentiate between pilocytic astrocytoma and diffused ones (Losmanand Kaelin, 2013). In another study, IDH_1 is a sufficient marker that allows a better separation of primary GBM from other malignant astrocytoma than any other marker and will help to define more accurately this tumor entity in upcoming studies. The low number of primary GBM exhibiting IDH mutations in our series indicates that our sample set consists indeed predominantly of these tumors. IDH_1 mutations in GBM were found in general in younger patients and were associated with a better prognosis (Combs *et al.*, 2011; Kurian *et al.*, 2013).

We have also shown in this study that changes in IDH_1 expression in malignant astrocytoma (III and IV) and malignant oligodendroglioma tumors are indeed observed. Thus IDH_1 could be a candidate biomarker in glioma tumors. Thirant *et al.* in 2011 showed that proteomic analysis reveal an unexpected alteration in the expression of certain protein networks in IDH mutated oligodendroglioma. Four IDH_1 mutations in oligodendroglioma were analyzed with 2D-electrophoresis and *in silico* methods and revealed global proteomes that were very similar for all four cases (Thirant *et al.*, 2011; Basanta *et al.*, 2011). It is speculated that IDH mutants can serve as diagnostic and prognostic markers (Guo *et al.*, 2011).

Due to the wide variation in pI and molecular weight of IDH, in a variety of malignant gliomas, we propose this enzyme as being a candidate biomarker for the diagnosis and prediction of glioma tumors. Hopefully in the future, by examining the types of glioma biomarker candidates, we will be able to achieve a more unified and functional approach.

Abbreviations

ACN: acetonitrile; EPM: energy protein malnutrition; GBM: Glioblastoma Multiforme; IDH: isocitrate dehydrogenases; IPG: immobilized pH gradient; MALDI: matrix assisted laser desorption ionization; TIFF: tagged image file format; SDS: sodium dodecyl sulfate; TOF: time of flight; WHO: world health organization; 2DG: two-dimensional gel.

References

Atai, N.A., Renkema-Mills, N.A., Bosman, J., Schmidt, N., Rijkeboer, D., Tigchelaar, W., Bosch, K.S., Troost, D., Jonker, A., Bleeker, F.E., Miletic, H.,Bjerkvig, R., De Witt Hamer, P.C., and Van Noorden, C.J. (2011). Differential activity of NADPH-producing dehydrogenases renders rodents unsuitable models to study IDH1R132 mutation effects in human glioblastoma. J. Histochem. Cytochem. 59, 489-503.

Basanta, D., Scott, J.G., Rockne, R., Swanson, K.R., and Anderson, A.R., (2011). The role of IDH1 mutated

tumour cells in secondary glioblastomas: an evolutionary game theoretical view. Phys Biol. 8, 015016.

Bleeker, F.E., Atai, N.A., Lamba, S., Jonker, A., Rijkeboer, D., Bosch, K.S., Tigchelaar, W., Troost, D., Vandertop, W.P., Bardelli, A., and Van Noorden, C.J.,(2010). The prognostic IDH1(R132) mutation is associated with reduced NADP+-dependent IDH activity in glioblastoma. ActaNeuropathol. 119, 487-494.

Borodovsky, A., Salmasi, V., Turcan. S., Fabius, A.W., Baia, G.S., Eberhart, C.G., Weingart, J.D., Gallia, G.L., Baylin, S.B., Chan, T.A., and Riggins, G.J.,(2013).5-azacytidine reduces methylation, promotes differentiation and induces tumor regression in a patient-derived IDH1 mutant gliomaxenograft. Oncotarget. 4, 1737-1747.

Capper, D., Weissert, S., Balss, J., Habel, A., Meyer, J., Jäger, D., Ackermann U., Tessmer, C., Korshunov, A., Zentgraf, H., Hartmann, C., and von Deimling, A.,(2010).Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain Pathol. 20, 245-254.

Combs, SE., Rieken, S., Wick, W., Abdollahi, A., von Deimling, A., Debus, J., and Hartmann, C., (2011). Prognostic significance of IDH-1 and MGMT in patients with glioblastoma: one step forward, and one step back? RadiatOncol. 6,115.

Dang,L., White, D.W., Gross, S., Bennett, B.D., Bittinger, M.A., Driggers, E.M., Fantin, V.R., Jang, H.G., Jin, S., Keenan, M.C., Marks, K.M., Prins, R.M., Ward, P.S., Yen, K.E., Liau, L.M., Rabinowitz, J.D., Cantley, L.C., Thompson, C.B., Vander Heiden, M.G., and Su, S.M., (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462, 439-444.

Garber, K., (2010). Oncometabolite? IDH_1 discoveries raise possibility of new metabolism targets in brain cancers and leukemia. J Natl Cancer Inst. 102, 926-928.

Garrett-Bakelman, F.E., andMelnick, A.M., (2013). Differentiation therapy for IDH1/2 mutant malignancies. Cell Res.23, 975-977.

Guo, C., Pirozzi, C.J., Lopez, G.Y., and Yan, H., (2011). Isocitrate dehydrogenase mutations in gliomas: mechanisms, biomarkers and therapeutic target. CurrOpin Neurol. 24, 648-652.

Huse, J.T., Nafa, K., Shukla, N., Kastenhuber, E.R., Lavi, E., Hedvat, C.V., Ladanyi, M., and Rosenblum, M.K., (2010). High frequency of IDH-1 mutation links glioneuronal tumors with neuropil-like islands to diffuse astrocytomas.ActaNeuropathol. 122, 367-369.

Jansen, M., Yip, S., and Louis, D.N., (2010). Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers. Lancet Neurol. 9, 717-726.

Kim, W., and Liau, L.M., (2012). IDH mutations in human glioma. Neurosurg Clin N Am. 23, 471-480.

Krell, D., Assoku, M., Galloway, M., Mulholland, P., Tomlinson, I., and Bardella, C., (2011). Screen for IDH1, IDH2, IDH3, D2HGDH and L2HGDH mutations in glioblastoma. PLoS One. 6, e19868.

Kurian, K. M., Haynes, H. R., Crosby, C., Hopkins, K., and Williams, M., (2013). IDH mutation analysis in gliomas as a diagnostic and prognostic biomarker. Br J Neurosurg. 27, 442-445.

Losman, J. A., and Kaelin, W. G. Jr., (2013). What a difference a hydroxyl makes: mutant IDH, (R)-2-hydroxyglutarate, and cancer. Genes Dev. 27, 836-852.

Marie, S. K., and Shinjo, S. M., (2011). Metabolism and brain cancer. Clinics (Sao Paulo). 66, 1, 33-43.

Parsons, D.W., Jones, S., Zhang, X., Lin, J.C., Leary, R.J., Angenendt, P., Mankoo, P., Carter, H., Siu, I.M., Gallia, G.L., Olivi, A., McLendon, R., Rasheed, B.A., Keir, S., Nikolskaya, T., Nikolsky, Y., Busam, D.A., Tekleab, H., Diaz, L.A. Jr., Hartigan, J., Smith, D.R., Strausberg, R.L., Marie, S.K., Shinjo, S.M., Yan, H., Riggins, G.J., Bigner, D.D., Karchin, R., Papadopoulos, N., Parmigiani, G., Vogelstein, B., Velculescu, V.E., and Kinzler, K.W.,(2008).An integrated genomic analysis of human glioblastomamultiforme. Science. 321, 1807-1812.

Reitman, Z.J., Olby, N.J., Mariani, C.L., Thomas, R., Breen, M., Bigner, D.D., McLendon, R.E., and Yan, H., (2010). IDH1 and IDH2 hotspot mutations are not found in canine glioma. Int J Cancer. 127, 245-246.

Reitman, Z.J., and Yan, H., (2010). Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism. J Natl Cancer Inst. 102, 932-941.

Sonoda, Y., and Tominaga, T., (2010). 2-hydroxyglutarate accumulation caused by IDH mutation is involved in the formation of malignant gliomas. Expert Reveurother. 10, 487-489.

Takahashi, Y., Nakamura. H., Makino, K., Hide, T., Muta, D., Kamada, H., and Kuratsu, J.I., (2013). Prognostic value of isocitrate dehydrogenase 1, O6-methylguanine-DNA methyltransferase promoter methylation, and 1p19q co-deletion in Japanese malignant glioma patients. World J SurgOncol. 11, 284.

Thirant, C., Varlet, P., Lipecka, J., Le Gall, M., Broussard, C., Chafey, P., Studler, J.M., Lacombe, J., Lions, S., Guillaudeau, A., Camoin, L., Daumas-Duport, C., Junier,M.P., and Chneiweiss, H.,(2011). Proteomic analysis of oligodendrogliomasexpressing a mutant isocitrate dehydrogenase-1. Proteomics. 11, 4139-4154.

Turcan, S., Rohle, D., Goenka, A., Walsh, L.A., Fang, F., Yilmaz, E., Campos, C., Fabius, A.W., Lu, C., Ward, P.S., Thompson, C.B., Kaufman, A., Guryanova, O., Levine, R., Heguy, A., Viale, A., Morris, L.G., Huse, J.T., Mellinghoff, I.K., and Chan, T.A., (2012). IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature. 483, 479-483.

Xu, X., Zhao, J., Xu, Z., Peng, B., Huang, Q., Arnold, E., and Ding, J., (2004). Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity. J Biol Chem. 279, 33946-33957.

Yan, H., Bigner, D.D., Velculescu, V., and Parsons, D.W., (2009). Mutant metabolic enzymes are at the origin of gliomas. Cancer Res. 69, 9157-9159.

Yan, H., Parsons, D. W., Jin, G., McLendon, R., Rasheed, B.A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G.J., Friedman, H., Friedman, A., Reardon, D., Herndon, J., Kinzler, K.W., Velculescu, V.E., Vogelstein, B., and Bigner, D.D.,(2009).IDH1 and IDH2 mutations in gliomas. N Engl J Med. 360, 765-773.

Zhang, C., Moore, L.M., Li, X., Yung, W.K., and Zhang, W., (2013). IDH1/2 mutations target a key hallmark of cancer by deregulating cellular metabolism in glioma. NeuroOncol. 15, 1114-1126.