



Beta Actin Expression Profile in Malignant Human Glioma Tumors

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Abstract

Background: Proteomics is considered a new era in neurophysiological/ neuropathological research including brain tumors. Gliomas which are derived from glial cells are the most common type of brain tumor in humans.

Methods: In the present study the total protein content of healthy cells of the brain and brain tumor cells was extracted, purified and quantified by Bradford assay. Two-dimensional electrophoresis were used for protein separation followed by statistical analysis. Primary protein detection was performed based on the differences in isoelectric pH, molecular weight of proteins and protein data banks, which was further confirmed by MALDI-TOF/TOF mass spectrometry (MS).

Results: Our results showed elevated levels of beta-actin protein expression in glioma brain tumor cells. It is important to know when a cell is transformed and when it becomes malignant. Here we evaluated the beta-actin expression in malignant cells.

Conclusion: Since structural changes are highly involved in tumor cell malignancy, beta-actin elevations can contribute in glioma tumor cell invasiveness.

Keywords: Beta-Actin; Glioma; Proteomics; Mass Spectrometry.

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Introduction

Cancer is defined as an abnormal growth and replication of cells, which interferes with normal physiology and function of the tissues and the body.¹ Brain tumors are also a mass of unnecessary cells growing in the brain, which can be divided into two main groups. Primary brain tumors: tumors that originate from brain's tissues. Secondary brain tumors: which originate from other organs in the body and migrate to brain following metastasis.²⁻⁴

Primary brain tumors are classified as benign and malignant. Benign tumor cells have a slow proliferation rate, are rarely invasive with frequently normal microscopic phenotype. Malignant brain tumors on the other hand, are invasive with high proliferation rate and abnormal phenotype. Another classification by WHO consists of four tumor grades (I, II, III and IV).⁵⁻⁷

Glioma is the most common and most fatal type of primary brain tumors, which affects brain's hemispheres, but can also spread to brain parenchymal tissue. Glioma tumors are derived from glial cells and are sub-divided into astrocytoma, oligodendroglioma and oligoastrocytoma.^{8,9}

Proteomics studies can be useful both in biological and clinical research, while one of its main applications

is in tumor biology, which includes identifying possible protein biomarker candidates, diagnosis and treatment, pharmaceuticals, protein complex analysis and drug target identification and studying the mechanism of action and possible side effects of anticancer drugs. Following genomics studies, proteomics also help obtain protein expression profiles by using two-dimensional electrophoresis (2DE), mass spectrometry (MS) and Bioinformatics tools.¹⁰⁻¹²

In the present study, used 2DE for separated proteins, MALDI-TOF/TOF for identified, and clustering and principal component analysis (PCA) for investigated the bata-actin expression change, is performed to obtain beta-actin protein expression profile in malignant human glioma tumor cells.

Materials and Methods

Twelve malignant human glioma tumor tissues were collected from excess tumor after surgical resection at hospital and tumors grade and malignancy was determined by the pathology department at the same hospital. Two normal tissue samples were also collected from the safe zone margin of tumor tissues. All collected samples were stored at -80°C.

Protein Extraction

The following protocol was used for protein extraction and preparation from tumor tissue samples: tissue samples were washed by PBS, cell lysis by sonication (samples received 3 rounds of sonication and each round lasted 30 seconds), acetone (50% and 100%) wash at 4°C, 15000 g, and three rounds of 30 minutes each. In the following, the obtained pellet was kept on -20°C overnight. Protein solubilization following acetone removal by adding 1ml of rehydration buffer and 50 µL of protease inhibitor to each protein pellet containing tubes. Finally, the Bradford assay was used for analysis of each tissue samples protein content.

Two-Dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis for protein separation was performed with IPG strips (18 cm) by isoelectric focusing (IEF). IPG trips were then transformed to SDS-PAGE gel and proteins were further separated based on their molecular weight. Followed by a final Coomassie blue staining of SDS-PAGE gel.

SDS-PAGE Scan and Bioinformatics Analysis

SDS-PAGE gels were scanned using scanner 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, followed by primary analysis of 2D images by Quantity One® software. The obtained scanned images of SDS-PAGE gels were further analyzed by Non-Linear Dynamics Progenesis Same Spot® software. After comparing the obtained 2D images with control samples, primary protein detection was performed based on the protein bands.

Mass Spectrometry Analysis

MS was used to confirm the early protein detection results obtained by analysis of 2D image analysis. The identify of differentially expressed proteins ($P < 0.05$ and fold > 2) was established using MALDI TOF TOF MS.

Statistical Analysis, Clustering and Principal Component Analysis

The obtained results were statistically analyzed by *t* test and SPSS (version 19) and $P < 0.05$ was considered statistically significant.

Protein spots with $P < 0.05$ were divided into 2 groups: increased and decreased protein expression groups. Then, clustering was used to identify the location of significantly significant spots, followed by PCA in determining the accuracy of the obtained results. Arithmetic cluster analysis was performed on two groups. Arithmetic cluster analysis employs correlation analysis to define if alterations in the levels of one individual protein are associated with alterations in the levels of a second protein across all samples (glioma and normal tissues). Arithmetic correlation algorithms are integral to the Progenesis Same Spots software (Nonlinear Dynamics v 3.0, 2008). Multiple areas on correlation coefficients between protein features were calculated by Progenesis Same Spots and the information visually represented in the form of a dendrogram.

Results

Separated Protein Spots Analysis

Non-Linear Dynamics Progenesis Same Spot® software identified 243 spots (Figure 1A), from which 189 spots had $P < 0.05$ that contained 78% of all the spots. From these 189 spots, 117 spots showed differential expressions more than 2 fold. These 117 spots constitute 72% of the spots with $P < 0.05$ and 48% of the total spots. Differential

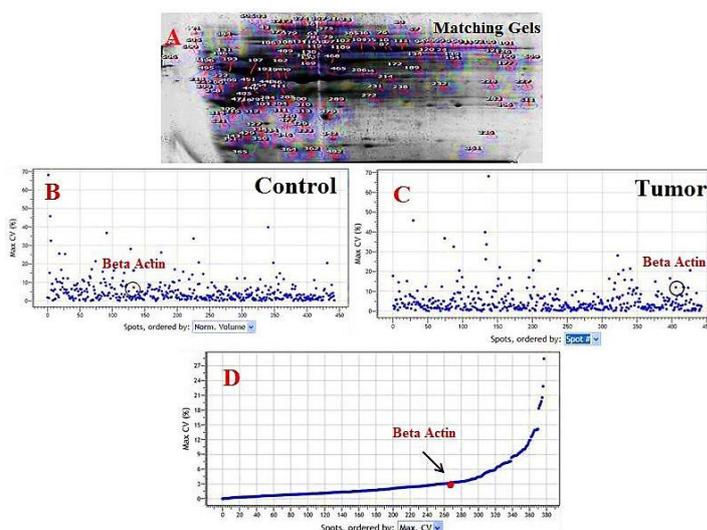


Figure 1. (A) Two-dimensional Gel Electrophoresis Image, (B) Beta Actin Proteins Position Alteration in Normal Glioma Tumor Tissue. (C) Changes of the Beta-Actin Protein Expression in Glioma Tumor Compared to Normal Tissue.

fold expression of all the spots with $P < 0.05$ are as follow: 79 spots with less than 2 fold (42% of the spots with $P < 0.05$ and 33% of the total spots). Sixty-one spots with 2-4 fold (32% of the spots with $P < 0.05$ and 25% of the total spots). Forty-nine spots with folds more than 4 (26% of spots with $P < 0.05$ and 20% of the total spots). Twelve spots corresponding to beta-actin were identified with elevated protein expression compared to normal tissue. These Twelve spots and their information are presented in Table 1. In Figure 1B and 1C, the position and position of beta-actin is shown on the gel (control and tumor). Also in Figure 1D, Increased expression of beta actin is shown in relation to the initial control.

Beta-Actin Protein Spot Confirmation by MALDI-TOF-TOF

Beta-actin protein spots were detected and confirmed by MALDI-TOF/TOF and the results are presented in Table 2.

Beta-Actin Protein Spot Confirmation by Clustering and Principal Component Analysis

As illustrated in Figure 2, spots located in the red and blue zone represent increased and decreased expression, respectively. Since the beta-actin spot is located in the red zone, it shows elevated protein expression. In order to continue to further understanding of rates of change, each of the tumors has been compared with the controls. Molecular weight and isoelectric pH values are recorded

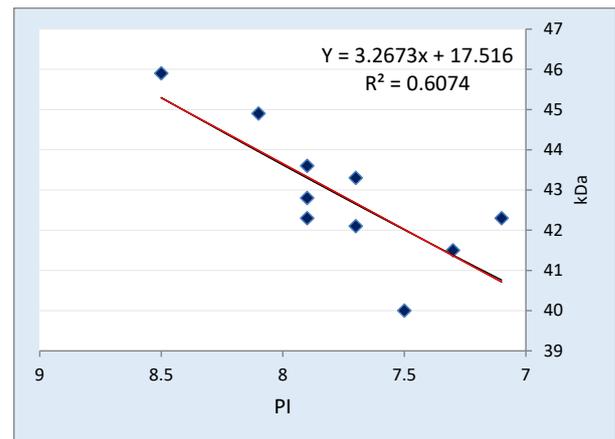


Figure 2. Diagram Scatter, Trandline Drawings for the Molecular Weight and Isoelectric pH, Based on Data From Table 1.

in Table 1, and the trend lines are shown in Figure 2. Afterwards, statistical analysis we have examined more closely, and it has been presented in Table 3 and Figure 3. PCA was also used to confirm the accuracy of clustering. PCA can be used to show that there are no variances in the data. PCA analysis results are presented in Figure 4.

Discussion

Since proteins are the cell's functional elements, studying their function and expression of transcription and even after their translation is important.¹³ Proteome

Table 1. Gel Electrophoresis Analysis and Information About Beta-Actin Protein Expression in Malignant Glioma Tumor Tissue

	Tumor	Grade	Sex	Age	Beta Actin			
					$P < 0.05$	Fold Change	PI	MW (kDa)
Case 1	Glioma-astrocytoma	III	Man	44	2.234e-013	+2.7	7.7	42.1
Case 2	Glioma-oligodendroglioma	III	Man	49	2.038e-009	+2.2	7.9	42.3
Case 3	Glioma-astrocytoma	III	Man	66	3.329e-009	+3.1	8.1	44.9
Case 4	Glioma-GBM	IV	Woman	59	2.586e-009	+4.1	8.5	45.9
Case 5	Glioma-astrocytoma	III	Man	38	2.310e-009	+3.5	7.1	42.3
Case 6	Glioma-astrocytoma	III	Woman	51	4.407e-009	+2.2	7.5	40.0
Case 7	Glioma-oligodendroglioma	III	Man	47	2.182e-009	+2.1	7.9	42.8
Case 8	Glioma-oligodendroglioma	III	Man	63	5.968e-009	+2.5	7.7	43.3
Case 9	Glioma-astrocytoma	III	Man	69	3.253e-011	+3.3	7.9	43.6
Case 10	Glioma-astrocytoma	III	Woman	59	1.876e-008	+2.3	7.3	41.5
Case 11	Glioma-GBM	IV	Man	56	2.942e-008	+3.6	7.4	44.4
Case 12	Glioma-GBM	IV	Man	60	7.806e-007	+3.5	8.1	43.9

Table 2. Beta Actin Protein Matching the Same Set of Peptides by Databank

	Expressed Proteins Change	Fold Change	Number of Peptides	Score	Matches	Sequences Coverage
Oligodendroglioma (III)	Up-Regulated	2.1-2.5	18	177	11/23	33
Astrocytoma (III)	Up-Regulated	2.3-3.5	29	201	19/24	21
Astrocytoma (IV)	Up-Regulated	3.5-4.1	21	189	18/20	31

Table 3. Statistical Analysis of Molecular Weight and Isoelectric pH for Beta Actin

	Valid	Missing	Mean	Median	Error of Mean	Variance	Min	Max	Rande
PI	12	0	7.76	7.8	0.39	0.15	7.1	8.5	1.4
MW	12	0	43.08	43.05	1.60	2.57	40	45.9	5.9

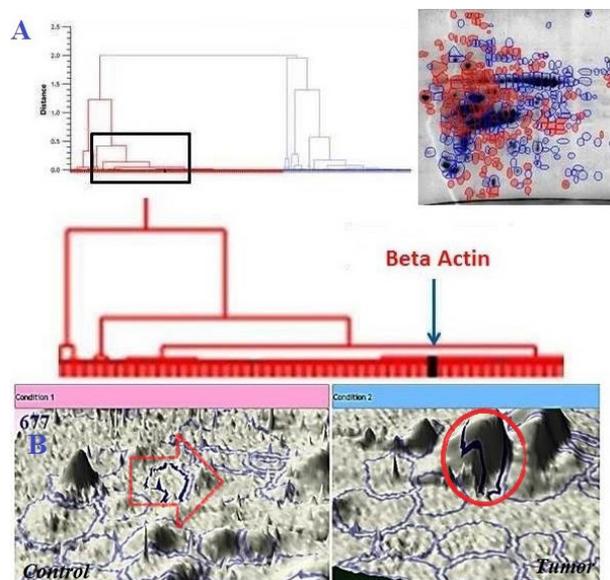


Figure 3. (A) Clustering the Proteins of Healthy and Tumor Sample Groups Based on Expression Changes. All these spots show significant expression changes in tumor tissue ($P < 0.05$ and fold > 2). Red and blue zone represent increased and decreased expression, respectively. (B) 3D Images of Btaactin Protein.

(the whole protein content of a genome) is related to different diseases. Studying the proteome is critical in order to achieve a novel system based on proteins for disease prognosis, prevention and treatment. Cancer cells

proteome analysis (including the study of biochemical and biophysical properties of proteomic profiles) which enables us to determine the role of different proteins in cancer initiation.^{14,15}

Proteomics studies are performed towards different goals including functional studies and finding novel protein biomarkers for rapid diagnosis. This study was performed with the aim of finding possible protein biomarkers for early glioma tumor detection.^{16,17} Our results showed elevated expression levels of beta-actin in malignant glioma tumors. Beta-actin is a very common skeletal protein that is present almost everywhere in the cell and is involved in cellular movements. Beta-actin's role in cellular movements affects other cellular functions as well including cell division, immune response and cellular transformation.¹⁸⁻²¹ Based on beta-actin's importance in cell function, proteomics investigations can be useful in better understanding and finding a biomarker candidate for malignant glioma. It is important to know when a cell is transformed and when it becomes malignant.^{22,23} Here we evaluated the beta-actin expression in malignant cells. Our results indicate that beta-actin expression control is controlled intracellularly since its expression levels differ with cell type and situation. Previous studies have indicated that tumor cell's malignancy is related to actin polymers. Despite no standard has been determined yet, all types of tumor cells show common cellular movements involving beta-actin protein.²⁴

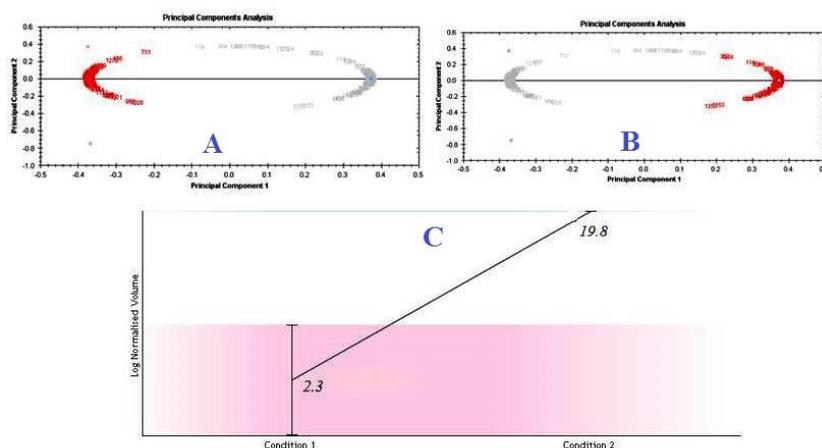


Figure 4. (A) Principal Component Analysis of Proteins From Healthy and Tumor Tissue Based on Expression Levels. All these spots show significant expression changes in tumor tissue ($P < 0.05$ and fold > 2). (A) Elevated expression compared to healthy tissue and (B) lower expression in tumor compared to healthy tissue. (C) For example: Beta actin protein has an up-regulation about 4.1 (fold = 4.1) in glioma-GBM (IV) brain tumors than normal brain tissue.

Nebl and colleagues studies on beta-actin showed that cell's skeletal proteins including actin fibers affect lipid rafts in the cell membrane. Lipid rafts play an important role in the membranes fluidity which in turn affects cellular pathogenicity.^{25,26}

Proteomics analysis has identified several proteins involved in oxidative stress response. Beta-actin is an abundant important protein in the glutathionylation process following oxidative stress. The first cellular response results in superoxide generation which can eventually results in cellular transformation.²⁷⁻³⁰

A cell needs to undergo specific structural changes in order to become malignant. Increase in beta-actin levels is related to invasiveness, including its effects on the carcinogen, primary and secondary GBM.^{31,32} In this situation, beta-actin polymerization results in an invasive structure which affects cellular movements and can lead to invasiveness and malignancy.^{33,34}

There are a large number of studies in the literature on beta-actin's role in different types of cancer and gliomas, including the studies of Fages et al, Ziv-Av et al and Panopoulos and colleagues which are all confirm our results on the effect of increased beta-actin expression in different types of cancer including glioma. However, different folding is reported by these groups and us.³⁵⁻³⁷

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Ethical Statement

All patients gave informed consent before participating in this study. The research was approved by the ethics committee of Islamic Azad university.

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