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The Investigation of Alpha-Tubulin Differential Expression in Oligodendroglioma Brain Tumor Aiming MALDI-TOF-TOF

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Abstract

Background: Tubulin is known as a heterodimer protein, which includes alpha and beta tubulin subunits. This structural protein plays important roles in pathogenesis and healing different diseases. Biomarkers help in fast and accurate detection of cancer. Proteomic studies can be useful both in biological and clinical research, also help obtain protein expression profiles by using two-dimensional electrophoresis, mass spectrometry, and bioinformatics tools. Finding candidate proteins as cancer biomarkers is an interesting area in proteomic investigations.

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

Results: In this study, an alpha-tubulin expression found changed (overexpression) in Oligodendroglioma tumors comparing control identified by proteomics analysis. Also, alpha-tubulin position showed in the oligodendroglioma tumors cluster diagram.

Conclusion: Proteome analysis approach has allowed biology and medical studies. Alpha-tubulin introduced as a candidate biomarker for the diagnosis and prediction of oligodendroglioma tumors.

Keywords: Alpha-Tubulin; Oligodendroglioma; MALDI-TOF-TOF; Proteomics.

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Introduction

Oligodendroglioma, a subtype of gliomas, account for 4.2% of all primary brain tumors.[1,2] Gliomas are the most malignant of human brain tumors, and also the most common type of primary brain tumor.[3-6] Oligodendroglioma tumors have a high sensitivity to chemotherapy; usually, they respond positively to the treatment.[7,8] The relevant genes and proteins conveying the favorable clinical effects associated with of 1p, 19q and oligodendroglioma tumors are still unknown.[9-11]

Tubulin is a heterodimer protein (100 Da), consisting of microtubules (alpha and beta) polypeptide chains. In human, alpha tubulin has 6 subunit genes, and also beta-tubulin has seven subunit genes, that expressed in different types of tissues.[12,13] However, in the nucleation of microtubules in a eukaryotic cell, γ-tubulin has a function. γ-Tubulin is a major protein in eukaryotic cells, that present and effective in centrosome and cytoplasm.[14] The structure of alpha and beta tubulin is highly dynamic.[15,16] Microtubules are critically involved in cellular processes, mitosis, intracellular transport, the scheduled cell death (apoptosis), the mechanism of cancer formation, and also cancer treatment (chemotherapy, radiation therapy, and anti-tumor drugs).[17,18] These microtubules has primary and essential functions in the cytoskeleton and may provide an attachment site for contractile proteins. Microtubules are cytoskeletal hollow fibers present in most eukaryotic cells that have cancer treatment users. Tubulin acetylated in ε amino, that conserved lys_{40}.[19-21] Previous reports demonstrated, intermediate filament proteins contain motifs (the motifs are short) that causes the connection of non-polymerizes tubules and that peptides include 24 amino acids, the ability and capacity binding to sites maintain tubulin.[22]

The proteomics research deals with systematic analysis of protein profiles of the proteins expressed in given cell, tissue, and biological system at a given time, and then compare the conditions differently.[23-25] As proven

in previous research, the proteome contains proteins resulting from the translation of the genome, as well as the proteins resulting from changes after translation. These are the backbone of protein biomarkers.[26,27] Cancer biomarkers for the early detection of malignancies and choice of appropriate treatment has requested in the different kinds of diseases.[28] Proteomics has produced types of biomarker candidates for cancer, But this is important, that biomarkers are specific.[29-31] The 2DE and MS are suitable methods for proteomics research, which analyzed the proteome of the glioma tissue and standard brain samples. Alteration in expression proteome detected, also showed most changes in expression levels of proteins related to metabolism, cytoskeleton, proteasome, and immune systems.[32,33] Advanced proteome imaging techniques included isoelectric focusing (IEF) approach, immobilized pH gradient (IPG) gel, gel trypsin digestion, and then MALDI-TOF-TOF analysis For proteins identification.[34,35]

In this article, we investigated the alpha-tubulin expression change in oligodendroglioma tumor. We extracted proteins and separated proteins using 2DE, analyzed the software, then identified alternation in oligodendroglioma of alpha-tubulin by statistical data, protein databases in proven research and MALDI TOF-TOF.

Methods

Patient Samples

Tissues were obtained, with informed consent and institutional review board approval, from patients undergoing tumor resection. For this study, all individuals filled a written informed consent form. Oligodendroglioma tumors were surgically removed and then classified [according to the World Health Organization (WHO)] at the hospital. The control tissue obtained from the margin of confidence around the tumor.

Tissue and Sample Preparation

The following protocol used for protein extraction and preparation of tumor tissue samples: tissue samples were washed with 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 kept on -20°C overnight. Protein solubilization following acetone removal by adding 1ml of rehydration buffer and 50 µL of a protease inhibitor to each protein pellet containing tubes. Finally, the Bradford assay used for the analysis of each tissue sample protein content.

Two-Dimensional Gel Electrophoresis

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

SDS-PAGE Scan and Bioinformatics analysis

We used Scanner [densitometer GS-800 (BioRad)] to scan gels. Followed by a primary analysis of 2D images by Quantity One® software. The obtained scanned images of SDS-PAGE gels has further analyzed by Non-Linear Dynamics Progenesis Same Spot® Software. After comparing the obtained 2D images with control samples, first protein detection was performed based on the protein bands.

SDS-PAGE gels were scanned using scanner Densitometer GS-800 (BioRad) scanner at 600 dpi in tagged image file format (TIFF). ImageMaster™ 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.

MS Analysis

MS was used to confirm the early protein detection results obtained by analysis of 2D image analysis. The identity of differentially expressed proteins was established using MS. The calculation standard in this study is fold more than two.

Statistical Analysis, Clustering, and PCA

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

Protein spots with $P<0.05$ 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. Cluster analysis performed on two groups.

1. Proteins that have increased expression
2. Protein that has decreased expression

Results

Electrophoresis and MALDI-TOF-TOF Analysis

We compared protein expression patterns between oligodendroglioma samples relative to control, using 2DGE and MALDI-TOF-TOF proteomics analysis. The 2DE showed 1328 spots. A total of 433 spots showed statistically significant differences in the image, alpha-tubulin (2 spots of total spots) were identified using the data obtained from MS in conjunction with a search of the databank (NCBI, www.ncbi.nlm.nih.gov/protein). MALDI-TOF-TOF results revealed that those two spots represent a total of up-regulated proteins. Among them the statistically significant protein spots alpha-tubulin proteins were definitely with pH_i: 4.94 and MW: 50804 Da detected which has an up-regulation about 3.1 (fold=3.1)

for the first spot, and (second spot) pH_i: 5.02 and MW: 50810 Da detected which has an up-regulation about 2 (fold=2) for the second spot, in oligodendroglioma tumors than control (Figure 1). Comparison of spot alpha tubulin between healthy tissue and tumor differentially expressed spot in oligodendroglioma tumor (Figure 2).

In this experiment, we saw changes in alpha-tubulin expression (up-regulated) in oligodendroglioma tumor than control identified by MALDI-TOF-TOF. Levels of alpha-tubulin spots were markedly higher in oligodendroglioma than non-tumor. We analyzed data from the MALDI-TOF-TOF, were shown in Table 1. Also, the position of the peptide presented has shown in Figure 3. Then, peptides that match the information in the databank searching obtained as shown in Table 2.

Spots Clustering

Spots statistical analysis have commonly used nonlinear Progenesis Samespots software in which the change in protein levels. Accordingly, proteins has classified into 2 categories: proteins that have increased expression and proteins that have reduced expression. Alpha-tubulin identified by MALDI-TOF-TOF, then it was identified in the expression increased group (red), alpha tubulin position in the clustering was determined by isoelectric pH and molecular weight. We have identifand alpha-tubulin position and was shown in Figure 4.

For more understanding about rates of change, each of the tumors has compared with the controls. MW and pH_i values recorded in Table 3. Then, statistical analysis has shown in Table 4.

Discussion

Proteomics and proteome analysis approach have allowed biology and medical studies of protein expression for different goals in different tissues, serum, CSF, urine and all biological fluids in despite conditions and types of Time.[36,37] For more understanding, the protein molecular mechanisms involved in rabies pathogenesis different proteomics approach has used including proteome analysis of several in vitro, and in vivo host models infected with

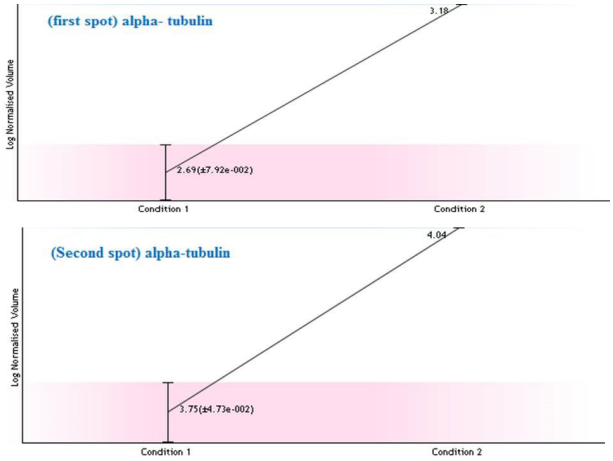

Figure 1. (First spot) alpha-tubulin protein has an up-regulation about 3.1 (fold=3.1) in oligodendroglioma brain tumor than normal brain tissue. (Second spot) alpha-tubulin protein has an up-regulation about 2 (fold=2) in oligodendroglioma brain tumor than normal brain tissue.

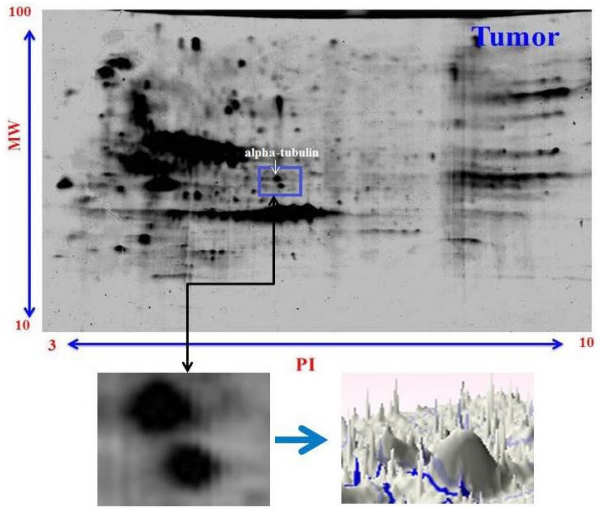

Figure 2. 3D Images of Alpha-Tubulin Protein in Oligodendroglioma Tumor Tissue.

Table 1. Analyzed Data by MALDI-TOF-TOF for Alpha-Tubulin in Oligodendroglioma Tumor Than Control

	Observed	Mr (expt)	Mr (calc)	PPM	Score	Expect
gi340021	1023.4638	1022.4565	1022.4417	14.5	58	0.0013
	1085.6414	1084.6341	1084.6128	19.6	69	8.6e-05
	1410.8043	1409.7970	1409.7667	21.5	96	1.9e-07
	1487.9089	1486.9017	1486.8719	20.0	93	2.4e-07
	1701.9344	1700.9272	1700.8985	16.8	119	7.6e-10
	1718.9132	1717.9059	1717.8747	18.2	60	0.00072
	1756.9852	1755.9780	1755.9559	12.5	121	5e-10
	2409.1929	2408.1856	2408.2012	-6.49	185	1.7e-16
gi37492	1457.8995	1456.8923	1456.8613	21.2	81	3e-06

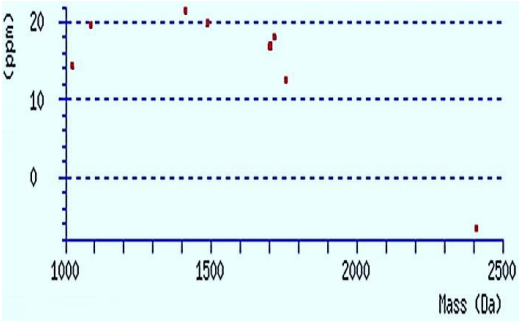

Figure 3. MALDI-TOF-TOF showed the position of the peptide for alpha-tubulin in oligodendroglioma tumor.

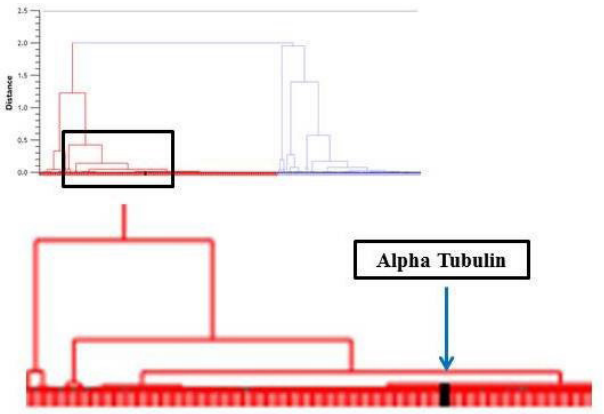

Figure 4. The alpha-tubulin position has shown in the diagram cluster.

different cancer related to protein, for example, dynamic alterations, phosphorylation, trafficking and localization, and protein-protein interactions. Phosphorylation and nuclear localization of protein cells involved in cancer may be clinical potential cancer biomarkers.[28,38] Actually, changes after translation such as acetylation and phosphorylation for tubulin is affecting the variety of diseases. These changes can be useful in diseases of the nervous system and neurodegenerative diseases. The acetylation of α tubulin plays an essential role in regulating stability and structure microtubule, specific tubulin function such as can division and intracellular trafficking.[39,40] The protein dynamic structures because of the interaction of alpha and beta tubulins polymers with associated microtubule. Microtubule has different functions in cells, such as cell motility, maintenance of cell shape, mitosis, and organization.[41,42]

Changes made to the amino acids cause may Cause the decrease of a specific contact with an interesting molecule.[43,44] The desire to change of a protein molecule or a lipid molecule depends on its basic structure and modifications of the peptide resulting from the sequence modification.[45] It is necessary to remember that small peptides line neurofilament (NFL) lightweight (about 61 kDa) tubulin binding sites. For example, the basic structure of tubulin is not known in complete detail, but protein baby related to the peptide [Gly-(Glu) 3-Gly-(Glu) 2 Y] that cause carboxy terminus of tyrosylated alpha-tubulin. It forms the ends of the amino acids (8 last amino acids) in alpha-tubulin.[46,47] The carboxy terminus of proteins structure, modifications peptide are essential for tubulin function.[48]

Alpha-tubulin can also be separated and identified by electrophoresis (IEF and SDS-PAGE) and spectroscopy (MALDI-TOF-TOF). The resulting examined by software, protein database, and tubulin isotype specific tryptic peptides identified. It is such important human tubulin in different isotype include α_3, α_4, α_6, α_8, $K_{\alpha 1}$, and $b_{\alpha 1}$. In all isotopes, post-translational modifications might create. α_4 tubulin was in the brain.[12,49] Alpha-tubulin isotype has a tyrosine or phenylalanine residue, which encoded at the end, but not exclusive. Researchers have shown that tyrosine accounts for a significant percentage of α_4 tubulin, That is the reason functional α_4 tubulin tyrosine ligase. The importance of the differences in tubulin can contribute to the biological recognition of the cytoskeleton system and the ways of rapid diagnosis and treatment of cancer.[12,20,50,51] Tubulin was tyrosinatation and acetylation (lys_{40}), was lower them the level of detection. Researchers have been investigating the correction of tubulin and post-transplantation in gliomas (astrocytoma and oligodendroglioma) and various types of cancer. [49]

We have shown changes in expression of proteins by clustering of alpha-tubulin related to oligodendroglioma; All the spots divided into 2 main clusters, Increase and

Table 2. Alpha-Tubulin Protein Matching the Same Set of Peptides By Databank Searching

	Tubulin Alpha	Mass	Score	Matches	Sequences
gi14389309	1C chain	50548	801	8(8)	8(8)
gi3474335	1B chain	50804	801	8(8)	8(8)
gi62897609	6 Variant	50476	801	8(8)	8(8)
gi193786502	Unnamed	46725	801	8(8)	8(8)
gi193787715	Unnamed	46825	801	8(8)	8(8)
gi296211572	1B chain isoform 2	46797	801	8(8)	8(8)
gi221039556	Unnamed	58636	799	8(8)	8(8)
gi6755901	1A chain	50788	789	8(8)	8(8)
gi73996547	1 isoform 9	46781	789	8(8)	8(8)

Table 3. The MW and pH_i of Each of the Tumors (Oligodendroglioma III) Compared to Control Independently

	Tumor	Grade	Sex	Age	Alpha-Tubulin		
					P < 0.05	MW	PI
Case 1	Oligodendroglioma	III	Woman	51	5.873e-008	51112	6
Case 2	Oligodendroglioma	III	Man	53	5.610e-006	52603	5.76
Case 3	Oligodendroglioma	III	Man	42	3.258e-008	48994	5.99
Case 4	Oligodendroglioma	III	Man	59	1.908e-005	51393	6.11
Case 5	Oligodendroglioma	III	Woman	58	7.654e-008	51507	5.98
Case 6	Oligodendroglioma	III	Woman	38	5.631e-005	53006	6.12
Case 7	Oligodendroglioma	III	Man	66	2.658e-007	49110	6.32
Case 8	Oligodendroglioma	III	Man	55	6.329e-006	48707	5.72

Table 4. Statistical Analysis of Molecular Weight and Isoelectric pH for Alpha-Tubulin

	Valid	Missing	Mean	Median	Error Of Mean	Variance	Min	Max	Rande
PI	8	0	6	6	0.182	0.033	5.72	6.32	0.6
MW	8	0	50804	51252.5	1563.82	2445543	48707	53006	4299

decrease expression, indicate that alpha tubulin placed in a cluster, which has an increased expression. This increase in alpha-tubulin expression is consistent with other studies, but with different overexpression. We have shown that alpha-tubulin as being a potential candidate for a biomarker of the diagnosis and prediction of oligodendroglioma tumors presented.

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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