

The Investigation of Heat Shock Protein (HSP70) Expression Change in Human Brain Astrocytoma Tumor

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Abstract

Background: Astrocytoma is the most common primary human brain tumor which has the most lethal hazard among tumors in human Central Nervous System. Heat Shock Proteins play an active role in cancer cells pathways through their effects on tumor cell proliferation, tumor differentiation, malignancy, metastasis, and cell death. HSP70 is a 70 KD protein that its expression change has been reported in various kinds of cancers. Here a proteomic activity for molecular diagnosis of Astrocytomas tumors is designed.

Methods: Proteins of tumor and normal brain tissues were extracted and then evaluated by Bradford test. In this study, the proteins were separated by 2DG Electrophoresis method, and then the spots were analyzed and compared using statistical data and specific software, after providing 3D images of spots alteration. Spots were identified by Isoelectric point (pI), molecular weights and data banks.

Results: As a result, the 2D gel showed totally 800 spots. HSP70 is one of down regulated proteins.

Conclusion: As conclusion, alteration of HSP70 expression was detected in Astrocytomas which has an important role in tumor survival and development, response to cell stress and induces apoptosis in cells. Three kinds of HSP70 are known in different cancers: HSP70-1, HSP70-2 and HSP70-8 which affect limitation of apoptosis system in cancer cells.

Keywords: HSP70 Heat-Shock Proteins; Astrocytoma; Proteomics; Electrophoresis

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Introduction

Proteomic approaches were most widely based on methods using differential expression on two Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE) gels [1-3]. Two Dimensional Gel Electrophoresis (2DGE) couples Isoelectric Focusing (IEF) in the first dimension and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) in the second dimension to separate proteins according to two independent parameters, pI in the first dimension and Molecular Weight (MW) in the

second dimension [4, 5]. The proteomic analysis techniques differentiate between normal brain tissues and astrocytoma tissues and also help to classify subtypes of biological aggressive astrocytoma [6, 7]. The ubiquity of these proteins was considered to arise from cellular stress responses and limitations of 2DGE [8-10]. Moreover, two other key areas of caution are successful application of MS and statistical analysis to ensure that proteins are correctly identified [5, 8]. According to their different molecular weights, structure and functions, HSPs contain major five groups, including HSP110/

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HSP70, HSP90 HSP70 HSP60 and small HSP families [11, 12]. Astrocytoma is the most common primary human brain tumor which has the most lethal hazard among tumors in human central nervous system [4, 13]. Astrocytomas are tumors in which neoplastic cells show distinct, albeit variable features of astrocytes [5, 14, 15]. Most brain Gliomas arise from astrocytes which are the most abundant type of glial cells, and are known as Astrocytomas [6, 7]. Malignant Gliomas are the most common human primary brain tumors. Glioblastoma Multiforme (GBM) is the most aggressive and lethal form [1, 3]. Heat shock Proteins (HSPs) are evolutionary conserved proteins involved in various cellular processes [16, 17]. HSPs, are highly conserved, function as molecular chaperons for a large panel of "Clint" proteins and have strong cytoprotective properties, induced by many different stress signals. They promote cell survival in adverse conditions. Therefore, their roles have been investigated in several conditions and pathology [18-19]. In the present study, HSP70 protein expression change in human brain Astrocytoma tumor is investigated by proteomic approaches.

Materials and Methods

Patient Samples

Astrocytoma tumors were surgically removed at Shohadaye Tajrish Hospital. The tumors were classified by neuropathologist team according to the guidelines of the World Health Organization (WHO) classification of tumors of the central nervous system. In accordance with laws, patients were informed and they allowed their tissue to be used in this study.

Tissue and samples preparation

Tissue samples of both tumor and normal brain tissue were snap-frozen immediately after operation in liquid nitrogen and stored at -80°

C until used for experimental analysis. The samples were broken into suitable pieces and were homogenized in lysis buffer II consisting of lysis buffer I (7M Urea, 2M Theourea, 4% CHAPS, 0.2% 100×Bio-Lyte 3/10), DTT, 1mM Ampholyte and protease inhibitor on ice. Cell lysis was completed by subsequent sonication (4×30 pulses). Samples were then centrifuged at 20000g at 4°C for 30 min to remove in soluble debris. The supernatants were combined with 100% aceton and centrifuged at 15000g and then the supernatants were decanted and removed (3 times). One hundred percent aceton were added to protein precipitant and kept at -20°C overnight. Samples were then centrifuged again at 15000g and the precipitant incubated 1h at room temperature. Protein samples were dissolved in rehydration buffer (8M urea, 1% CHAPS, DTT, Ampholyte SERVA-LYTE pH (4) and protease inhibitor). Protein concentrations were determined using Bradford test and Spectrophotometry method and the protein extracts were then separated and used for 2DG electrophoresis.

Two-dimensional gel electrophoresis

The isoelectric focusing for the first dimensional electrophoresis was performed using 18cm, pH 3-10 IPG strips. Samples were diluted in a solution containing rehydration buffer, IPG buffer and DTT to reach a final protein amount of 500µg per strip. Strips were subsequently subjected to voltage gradient as described in manufacturer's instruction. Once focused, IPG strips were equilibrated twice for 15min respectively in equilibration buffer I (50mM tris-HCl pH 8.8, 6M urea, 30% glycerol, 2% SDS and DTT) and equilibration buffer II. The second dimension SDS-PAGE was carried out using 12% polyacrylamide gels. Followed SDS-PAGE, gels were stained using coomassie blue method overnight (Figure1).

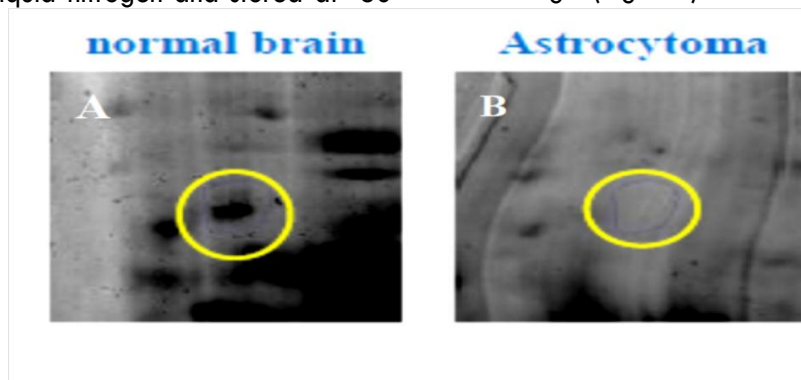


Figure 1. Images of HSP70 protein in **A)** normal brain tissue **B)** Astrocytoma tumor tissue

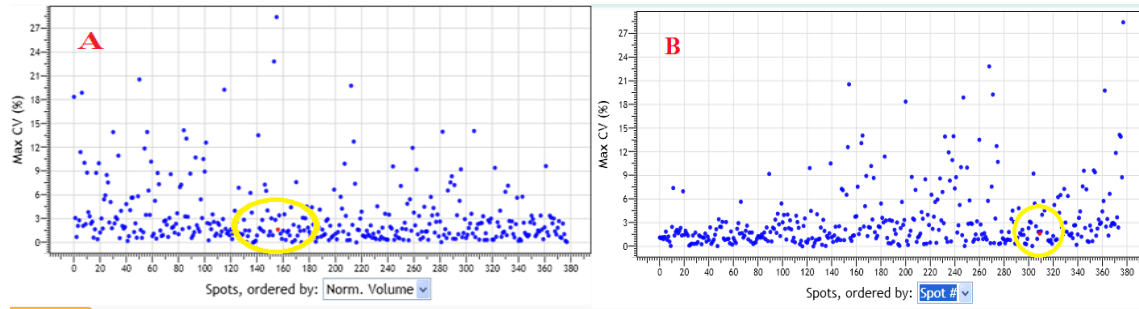


Figure 2. HSP70 protein position alteration in **A)** normal brain tissue **B)** Astrocytoma tumor tissue

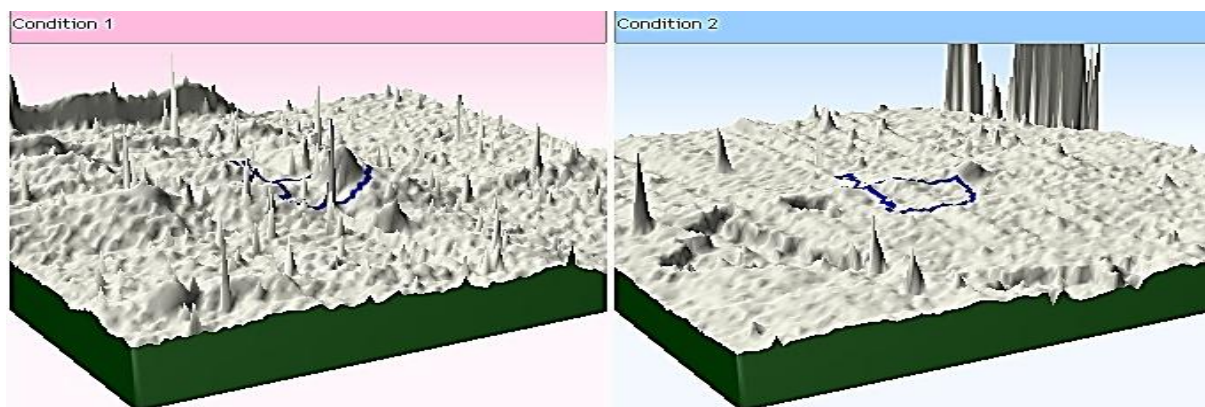


Figure 3. 3D images of HSP70 protein in **A)** normal brain tissue **B)** Astrocytoma tumor tissue

Image analysis

Gel images were analyzed by Progenesis Same spots software to identify spots differentially expressed between tumor and control samples based on their volume and density. Spots were carefully matched individually and only spots that showed a definite difference were defined as altered (Figure 2) after providing 3D images of spots alteration (Figure 3).

Results

Using 2D-PAGE proteomic analysis, we compared protein expression patterns between

Astrocytoma samples relative to control tissue. The 2D gel electrophoretograms revealed consistent protein profiles for each group. Simple statistical tests were used to establish a putative hierarchy in which the change in protein level were ranked according to a cut off point with $p < 0.05$. Among the statistically significant protein spots ($p < 0.05$) HSP70 protein was definitely with pI 5.04 and MW 71kD detected which has a down regulation about 2 (fold=2) in astrocytoma brain tumors than Normal brain Tissue (Figure 4 and 5).

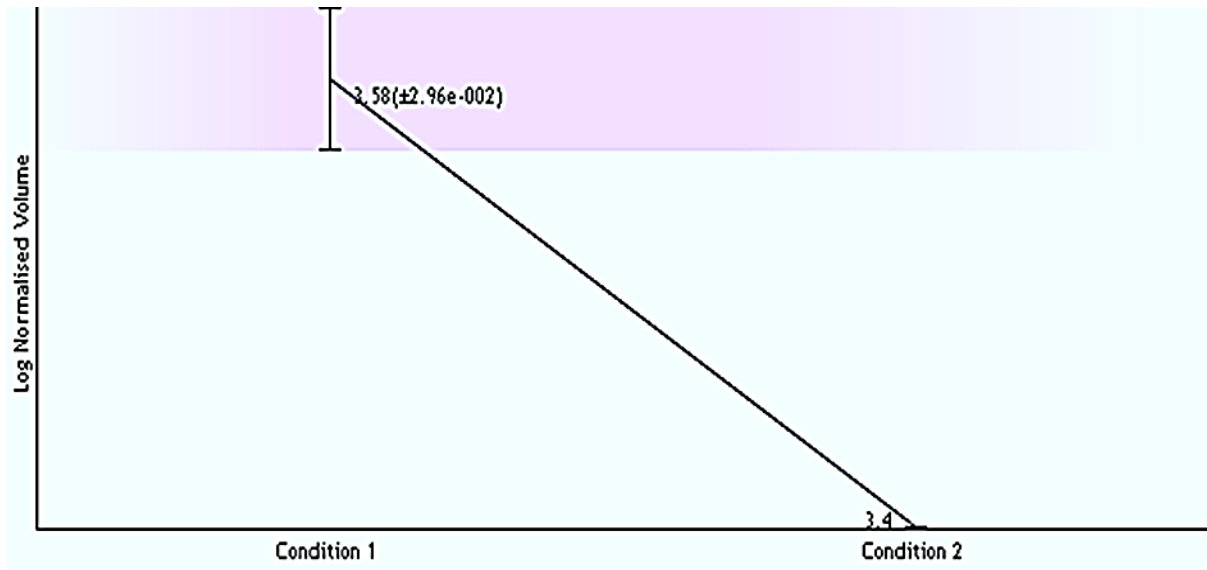


Figure 4. HSP70 protein has a downregulation about 2 (fold=2) in Astrocytoma brain tumor than normal brain tissue.

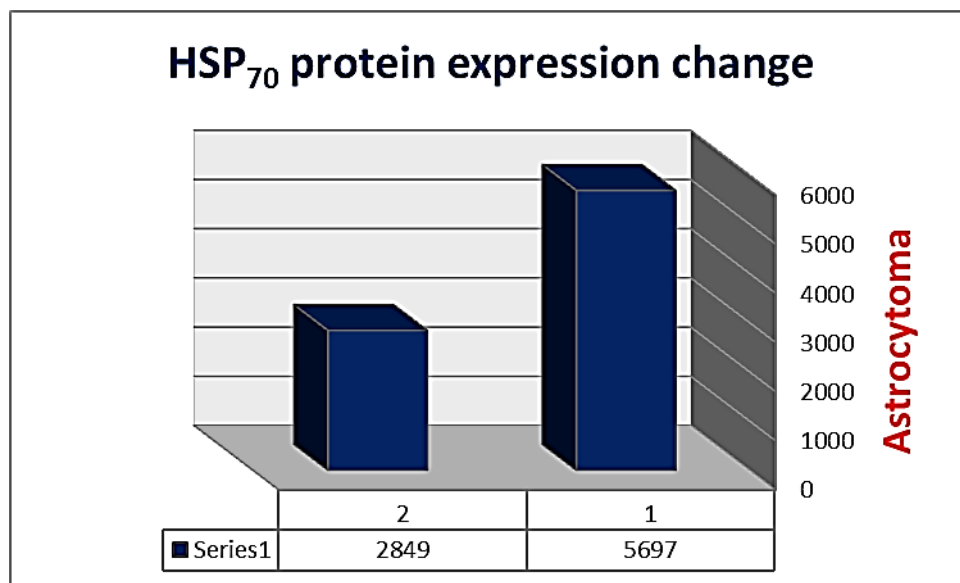


Figure 5. HSP70 protein expression chart: showing differential expression between the control (1) and Astrocytoma (2) (under expression)

Discussion

Glioblastoma multiforme and other grades of Astrocytoma proteomics has contributed significantly in Gliomas research to investigate disease pathobiology and identification of novel therapeutic targets and potential markers [1, 20 - 22]. The expression of HSPs is induced under the existence from oxidative stresses, heavy metals, osmotic stresses, metabolic poisons, viral/microbial infections. HSP70 is over expressed in a wide range of human

cancers and implicated in tumor cell proliferation, differentiation, invasion, metastasis, death and recognition by immune system [18, 23]. Among the diverse mammalian HSPs some members share several features that may qualify them as cancer biomarker [18 , 24]. HSP70 is a highly conserved protein that refolds misfolded proteins and has numerous housekeeping functions which regulate apoptosis and other cell activities [23-25]. HSP70 consist of a nucleotide binding domain which bind ATP and a substrate binding domain

that binds misfolded proteins. This protein is 70 kD protein that its expression change has been reported in various kinds of cancer [26-28]. Guang Zhang Xiong et al report, HSP70 play an important role in growth and invasively biological malignant Glioma characteristics. A total of four proteins (HSP27, alpha- β -crystalline, HSP70 and chaperonin) involved in physical tumor processes are significantly over expressed in low grade Astrocytomas. Protein over expression potentially relates to increased protein synthesis/degradation pathways in rapidly growing tumors [6, 7, 27]. Three kinds of HSP70 are known in different cancer: HSP70-1, HSP70-2 and HSP70-3 which affect limitation of apoptosis system in cancer cells. Leukemic and numerous other cancer cells have a greater amount of HSP70-1, HSP70-2, HSPA5, HSP27 and HSP90AB1 all had lower expression level in tumor than in normal tissue [11, 25]. Both HSPA9 and HSPA5 belong to the same HSP70 family. HSPA9 is increased in high Glioma tumors, whereas HSPA5 is decreased [11, 12]. HSP70 protein isoform 9, a major mitochondrial protein plays a central role in protein import and export. Overall, this work underlines the down regulation of Astrocytoma related proteins. This might be attractive clinical biomarker [1, 28].

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Conflict of Interest

The Authors have no conflict of interest in this article.

Authors' Contribution

Mostafa Rezaei-Tavirani, Solmaz Khaghani Razi Abad and Mehdi Pooladi designed the study, gathered and analyzed the data and wrote the paper. Sara Sobhi contributed to writing the manuscript. Mehrdad Hashemi, Masoumea Mousavi, Hakimeh Zali and Mona Zamanian Azodi contributed to study design. Afshin Moradi, Ali Reza Zali, Azadeh Rakhshan and Mehdi pooladi contributed to samples collection and indentation.

References

1. Collet B, Guitton N, Saikali S, Avril T, Pineau C, Hamlat A, et al. Differential analysis of Glioblastoma Multiforme proteome by a 2D-DIGE approach. *Proteome Science*. 2011; 9: 1-16.

2. Fang X, Wang C, Balgley BM, Zhao K, Eang W, He F, et al. Targeted tissue proteomic analysis of human Astrocytomas. *American Chemical Society*. 2012; 11(8): 3937-46.

3. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary Glioblastoma. *The American Journal of Patobiology*. 2007; 170(5): 1445-51.

4. Weiss W, Gorg A. High-Resolution Two-Dimensional Electrophoresis. *Proteomics methods in molecular biology*. 2009; 564: 13-20.

5. Deighton RF, Short DM, Gregor RJ Mc, Gow AJ, Wittle IR, Culloch J Mc. The utility of functional interaction and cluster analysis in CNS Proteomics. *Journal of Neuroscience Methods*. 2009; 180(2): 321-9.

6. Xiong G, Xiao H, Lu J, Zhang D, Bi C, Peng L, et al. Differential protein expression in low-grade Astrocytomas and peritumoral human brain tissues. *Neural Regeneration Research*. 2010; 5: 1915-20.

7. Seyfried NT, Huysentruyt LC, Atwood JA, Xia Q, Seyfried TN, Orlando R. Up-regulation of NG2 proteoglycan and interferon induced transmembrane proteins 1 and 3 in mouse Astrocytoma: A membrane proteomics approach. *NIH Public Access*. 2008; 263(2): 1-15.

8. Chen X, Zang W, Xue F, Shen Z, Zhang Q. Bioinformatics analysis reveals potential candidate drugs for different subtypes of Glioma. *Neurol Sci*. 2013; 34(7): 1139-43.

9. Cho WC. Contribution of oncoproteomics to cancer biomarker discovery. *Molecular Cancer*. 2007; 6: 1-13.

10. Khalil AA. Biomarker discovery: a proteomics approach for brain cancer profiling. *Cancer Sci*. 2007; 98(2): 201-13.

11. Shen G, Liang S, Xu Z, Zhou L, Xiao S, Xia X, et al. Downregulated expression of HSP27 in human low-grade Glioma tissues discovered by a quantitative proteomic analysis. *Proteome Science*. 2010; 8: 1-17.

12. Shirai K, Suzuki Y, Okamoto M, Wakatsuki M, Noda S, Takahashi T, et al. Influence of histological subtype on Survival after Combined Therapy of Surgery and Radiation in WHO grade III Glioma. *J Radiat Res*. 2010; 51(5): 589-94.

13. Wise S, Karus M, Faissner A. Astrocytes as a source for extracellular matrix molecules and cytokines. *Frontiers in Pharmacology*. 2012; 3: 1-4.

14. Huse JT, Holland EC. Targeting brain cancer: advances in the molecular pathology of malignant Glioma and Medulloblastoma. *Macmillan Publishers Limited*. 2010; 10: 319-31.

15. Quesada A, Prada F A, Aguilera Y, Espinar A, Carmona A, Prada C. Peripapillary Glial cells in the chick retina: a special Glial cell type expressing Astrocyte, radial Glia, Neuron, and Oligodendrocyte markers throughout development. *GLIA*. 2004; 46(4): 346-55.

16. Wilde I B, Brack M, Winget J M, Mayor T. Proteomic characterization of aggregating proteins after the inhibition of the ubiquitin

proteasome system. *J Proteome Res.* 2011; 10(3): 1062-72.

17. Zhang M, Wan C, Ji B, Zhang Z, Zhu H, Tian N, et al. Proteome alteration of U251 human Astrocytoma cell after inhibiting retinoic acid synthesis. *Mol Cell Biochem.* 2009; 323(1-2): 185-93.

18. Seigneuric R, Mjahed H, Gobbo J, Joly A-L, Berthenet K, Shirley S, et al. Heat Shock Proteins as danger signals for cancer detection. *Front Oncol.* 2011; 10: 3389-92.

19. Elstner A, Stockhammer F, Nguyen-Dobinsky TN, Nguyen QL, Pilgermann I, Gill A, et al. Identification of diagnostic serum protein profiles in Glioblastoma patients. *J Neurooncol.* 2011; 102(1): 71- 80.

20. Gollapalli K, Ray S, Srivastava R, Renu D, Singh P, Dhali S, et al. Investigation of serum proteome alterations in human Glioblastoma Multiforme. *Proteomics.* 2012; 12(4): 2378-90.

21. Somasundaram K, Nijaguna MB, Kumar D M. Serum proteomics of Glioma: methods and application. *Expert Review of Molecular Diagnostics.* 2009; 9(7): 695- 707.

22. Carlsson A, Person O, Ingvarsson J, Widegren B, Salford L, Borrebaeck CAK, et al. Plasma proteome profiling reveals biomarker

patterns associated with prognosis and therapy selection in Glioblastoma Multiforme patients. *Proteomics Clin Appl.* 2010; 4(6-7): 591-602.

23. Ciocca R, Calderwood S. Heat Shock Proteins in cancer: diagnostic, prognostic, predictive and treatment implications. *Cell Stress Chaperones.* 2005; 10(2): 86-103.

24. Sherman M, Multhoff G. Heat Shock Protein in cancer. *Ann N Y Acad Sci.* 2007; 1113: 192-201.

25. Hatfield MP, Lovas S. Role of HSP70 in cancer growth and survival. *Protein Pept Lett.* 2012; 19(6): 616-24.

26. Ericson C, Mister M. National biobank program workpackage brain proteomics biobanking. 2006; 5: 1-3.

27. Sun S, Wong T S, Zhang X Q, Jenny K S P, Lee N P, Day P J R, et al. Protein alterations associated with Temozolomide resistance in subclones of human glioblastoma cell lines. *J Neurooncol.* 2012; 107(1): 89- 100.

28. Vogel T W, Zhuang Z, Li J, Okamoto H, Furuta M, Lee YS, et al. Proteins and protein pattern differences between glioma cell lines and glioblastoma multiforme. *Clin Cancer Res.* 2005; 11(10): 3624-32.