Original Article

Rho GDP Protein Expression Change and Investigation of ItsEffect on GDP/GTP Cycle in Human Brain Oligodendroglioma

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Abstract

Background: Oligodendrogliomas are sub-types of Gliomas with an enriched net of branching capillary. A vast amount of Oligodendroglial (approximately 60-90%) demonstrates a loss of 1p and 19q chromosomes, and their response to radio and chemotherapy treatments are considerably more favorable and less aggressive. Rho GDP inhibitor is a protein which controls the activity and distribution of GTPase in cells. In cancer cells with 1pLOH deletion, Rho GDP inhibitor protein can be anti-apoptotic. Here understanding of molecular diagnosis of Oligodendroglioma tumors is investigated via proteomic tools.

Methods: Proteins of tumor and normal brain tissues are extracted and separated by Two-Dimensional Gel (2DG) Electrophoresis method and the spots were then analyzed and compared using statistical data and specific software, after providing 3D images of spots alteration. Spots were identified by pI, molecular weights and data banks.

Results: The 2D gels of normal and patient were provided and compared. As a result,high resolution analysis showed that there are totally 1328 spots in the gel. Bioinformatic analysis of the gels revealed that Rho GDI is one of the downregulated proteins.

Conclusion: It was revealed that Rho GDP inhibitor deactivates GTP-Protein complex through disintegration of GTP from Rho proteins, leading to prevention of Rho GDP to return to its cycling.

Keywords: Rho GDP; Oligodendroglioma; Two-Dimensional electrophoresis; Proteomics; Brain tumors

Please cite this article as: Khaghani Razi Abad S, Pooladi M, Hashemi M, Moradi A, Zali A, Mousavi M, Zali H, Zamanian Azodi M, Rakhshan A, Vafaee R, Rezaei-Tavirani M, Sobhi S. Rho GDP Protein Expression Change and Investigation of ItsEffect on GDP/GTP Cycle in Human Brain Oligodendroglioma. Iran J Cancer Prev. 2013; 6(Suppl.):24-9.

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Received: 9 Jan. 2013
Accepted: 12 Feb. 2013
Iran J Cancer Prev 2013; 1(Suppl.):24-9

Introduction

Uncontrolled proliferation of glial cells result in the formation of tumors in brain, known as Glioma, which is considered as one of the prime causes of cancer related mortality [1-4].WHO classification and grading of the diverse type of Gliomas are primarily based on morpho-histological features of tumors. Thus far, only a few molecular parameters have gained clinical significance in diagnostic assessment of Gliomas.In oligodendroglial tumors, combined deletion of 1p/19q which is found in up to 80% of oligodendrogliomas and about 50% at anaplastic oligodendroglioma is the dominant parameter in oligodendroglial tumor diagnosis [5-7].

Gliomas are the most frequent primitive tumors of the human central nervous system, and have variable histological appearance. Among them Oligodendrogliomas are infiltrating diffuse tumor with annual incidence rate of 0.2 per 100000 people in Europe. They are predominantly a tumor of adulthood with a peak incidence between the ages of 45-50 years old[8-

10].Oligodendroglioma constitute 4% of all brain tumor and 5-20% of all glial tumors [11].Proteomics is increasingly employed in both neurological and oncological research to provide insight into the molecular basis of disease but rarely has a coherent, novel pathophysiological insight emerged [8].Oncoproteomics is the study of proteins and their interactions in a cancer cell by proteomics technologies. Proteomics research first came to the fore with the introduction of two-Dimensional Electrophoresis (2DE) [12].Two-Dimensional Electrophoresis (2DE) with Immobilized PH Gradients (IPGs) combined with protein identification by mass spectrometry is,currently the workhorse for the majority of ongoing proteome projects [13, 14]. Previously, 2D polyacrylamide gel electrophoresis coupled with mass spectrometry has been the primary proteomic technology used for biomarker discovery.Thisis well-studied technique for direct comparison of differentially expressed proteins between normal and tumor tissue [15-17].In the present study, we investigated the Rho GDI protein expression change in human brain Oligodendroglioma tumor to get an understanding of data and specific software molecular diagnosis of Oligodendrogliomas.Here proteins of tumoral and normal brain tissues are extracted and evaluated by proteomic tools. After providing 3D images of spots, their alteration are monitored using statistical data and specific software.Using different proteomics approaches multiple differentially expressed Oligodendroglioma proteins are identified, few of which could further be investigated as potential surrogate marker for Oligodendroglioma.

Materials and Methods

Patient samples

Oligodendroglioma tumors were surgically removed at Shohada Tajrish Hospital .The tumors were classified by neurophathologist 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 proteomic analysis. To obtain tissue extracts, 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 insoluble debris.The supernatants were combined with 100%acetone and centrifuged at 15000g and then the supernatants were decanted and removed (3 times). One hundred percentacetone were added to protein precipitant and kept at -20°C overnight. Samples were then centrifuged again at 15000g and the precipitant incubated 1hour at room temperature. Protein samples were dissolved in rehydration buffer(8MUrea, 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 isoelectricfocusing 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).

Image analysis

Gel images were analyzed by Progenesis Samespots 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 3D images of spots alteration were provided (Figure 3).

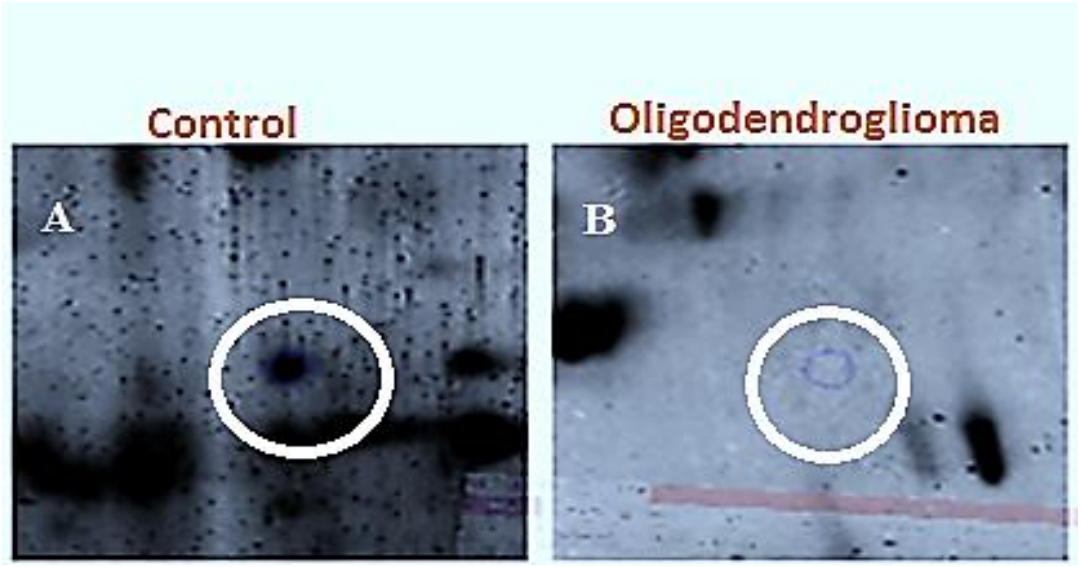

Figure 1. 2D gel images of Rho GDI protein in A) normal brain tissue B) tumor tissue

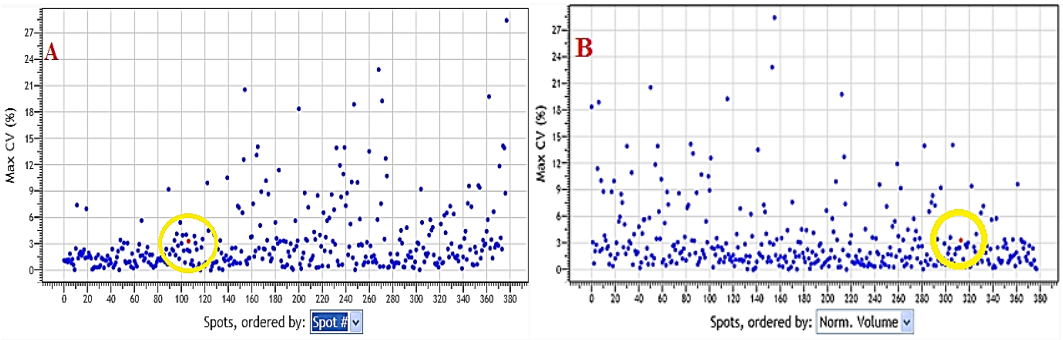

Figure 2. Rho GDP protein position alteration in A) normal brain tissue B) tumor tissue

Results

Using 2D-PAGE proteomic analysis, protein expression patterns between Oligodendroglioma samples relative to control tissue were compared.The 2D gel electrophoretograms 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 a cutoff point with $p<0.05$. The 2D gel showed totally 1328 spots among which 276 spots were under expressed and 157 spots were overexpressed. Among the statistically significant protein spots ($p<0.05$) Rho-GDP protein was definitely with pI 5.21 and MW 23.2 kD detected which has an downregulation about 3.7 (fold=3.7) in Oligodendroglioma brain tumors thannormal brain tissue (Figures 4 and 5).

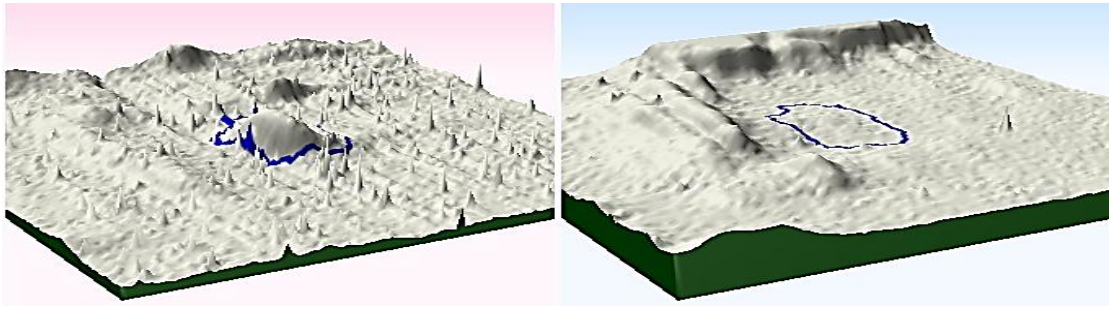

Figure 3. 3D images of Rho GDI protein in A) normal brain tissue B) tumor tissue

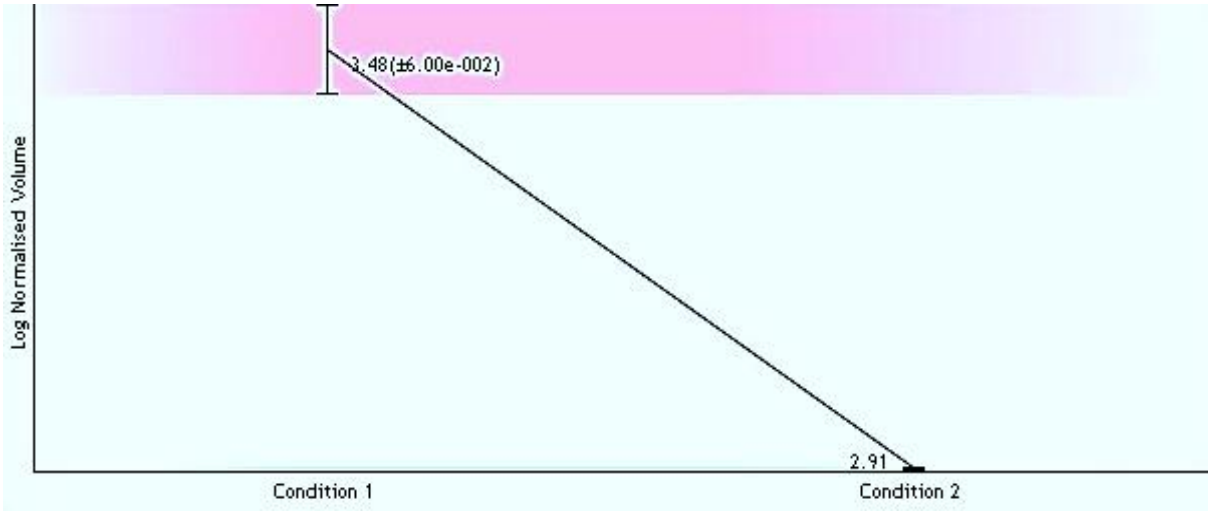

Figure 4. Rho-GDP protein has a downregulation about 3.7 (fold=3.7) in Oligodendroglioma brain tumors thannormal brain tissue.

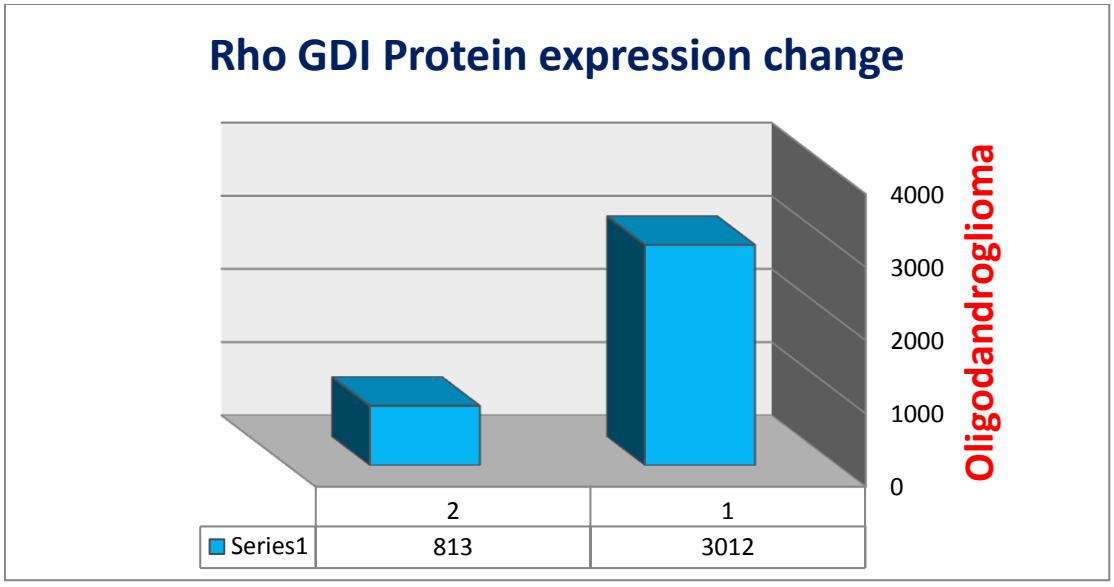

Figure 5. Rho GDP protein expression chart: showing differential expression between the Oligodendroglioma (2) and control (1) (under expression)

Discussion

Cancer stem/initiating cells are known to have the ability of self-renewal, multi-lineage differentiation and proliferation [18, 19].As an important biological indicator of cancer status, biomarkers represent powerful tools for monitoring the course of cancer and gauging the efficacy and safety of novel therapeutic agents [12, 20].In Gliomas, (and many other neurological disease and cancer), a glut of proteomic data have been generated but there has been no unitary approach to establish which key proteins and/or signaling pathways have been identified[8]. Oncoproteomics has the potential to revolutionize clinical practice including cancer diagnosis and screening based on proteomics platforms (a complement to histopathology in individualized selection of therapeutic combinations).Proteomic platforms target the entire cancer specific protein network, real-time assessment of therapeutic efficacy and toxicity and rational modulation of therapy based on changes in the cancer protein network associated with prognosis and drug resistance[12, 21].

Among the proteins screened for differential expression in Oligodendrogliomas, Rho GDP dissociation inhibitor (Rho GDI) controls the activity and distribution of GTPases in cells[16].

Rho GDIs are the negative regulators of the functional cycle of small GTP-binding proteins of the rho/rac/sdc42 family that couple a GDP/GTP cycle stimulated by guanine nucleotide exchange factors and terminated by GTPase-activating proteins. Rho GDI alpha is a representative member among all three rho GDIs found in mammals and it is able to form cytosolic complexes with most members of the rho family[22, 23].This protein have previously been shown to enhance chemo resistance in other tumors and it has been reported to be an anti-apoptotic protein in cancer cells and overexpressed in many types of human cancer. Rho GDI has been suggested to play a role in drug resistance in certain cancers such as melanoma and ovarian cancer and confirmsthat Rho GDI has an overexpression in Oligodendrogliomas without 1pLOH.It can therefore be another candidate protein for chemo resistance. Rho protein family members are known to involve in a number of biological processes, including cell transformation and adhesion. Underexpression of this protein can result in enhancement of its activities and uncontrolled Rho GDI functions are reported to be associated with carcinogenesis and aggressive behavior of cancer such as metastasis and invasion [24-26].Therefore, it can be said that Rho GDI protein is an important protein with multiple activities in Oligodendrogliomas which can be considered with two approaches, chemoresistency behavior and aggressive behavior or invasion in gliomas[23, 25].

Acknowledgment

This study was supported by Proteomics Research Center, ShahidBeheshti University of MedicalSciences and contributed by Department of Pathology,Shohada Hospital, Shahid Beheshti Universityof Medical Sciencesand Department of Neurosurgery, Shohada Hospital.

Conflict of Interest

The authors have no conflict of interest in this study.

Authors'Contribution

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

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