Gene 569 (2015) 77–82

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Proteomics analysis of human oligodendroglioma proteome

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ARTICLE INFO

Article history:
Received 24 April 2015
Received in revised form 8 May 2015
Accepted 10 May 2015
Available online 19 May 2015

Keywords:
Glioma
Oligodendroglioma
Proteomics
2-DG electrophoresis

ABSTRACT

Proteomics analyses enable the identification and quantitation of proteins. From a purely clinical perspective, the application of proteomics based on innovations, may greatly affect the future management of malignant brain tumors. This optimism is based on four main reasons: diagnosis, prognosis, selection of targeted therapy based on molecular profile of the brain tumor and monitoring therapeutic response, or resistance. We extracted the proteins of tumor and normal brain tissues, and then evaluated the protein purity by Bradford test. In this study, we separated the proteins by two-dimensional (2DG) gel electrophoresis methods. Then spots were analyzed, compared using statistical data and specific software and were identified by pH isoelectric, molecular weights and data banks. The protein profiles were determined using 2D gel electrophoresis and MALDI TOF/TOF mass spectrometry approaches. Simple statistical tests were used to establish a putative hierarchy in which the change in protein level was ranked according to a cut-off point with $p < 0.05$. The 2D gel showed a total of 1328 spots among which 157 spots were under-expressed and 276 spots were overexpressed. Most proteins are subjects to post-translational modifications, where amino acid residues may be chemically modified or conjugated by small proteins like ubiquitin. Proteomics is a powerful way to identifying multiple proteins which are altered following a neuropharmacological intervention in a CNS disease.

1. Introduction

Proteomics analyses enable the identification and quantitation of proteins (Lemee et al., 2013). The first objective of proteomic data mining is differential expression analysis. It consists of comparing two or more predefined biological conditions to accurately highlight the few protein spots of interest among noise (Rostomily et al., 2010). Current statistical methods give good results when data are correctly preprocessed. From a purely clinical perspective, the application of proteomics based innovations may greatly affect the future management of malignant brain tumors. This optimism is based on four main reasons: diagnosis, prognosis, selection of targeted therapy based on the molecular profile of the brain tumor and monitoring therapeutic response, or resistance (Boja and Rodriguez, 2011; Vilasi et al., 2013).

Gliomas are the most frequent primary brain tumors in adults. The World Health Organization (WHO) classification divides gliomas into three main sub groups, astrocytomas, oligodendrogliomas and mixed oligoastrocytomas, and differentiates between four malignancy grades (Huse and Holland, 2010; Zhuang et al., 2011; Pooladi et al., 2013; Hashemi et al., 2014). Gliomas are very rare subtypes of pineal region tumors, which are thought to rise from surrounding glial stromal. Oligodendrogliomas are diffusely infiltrating tumors, arising in white matter of cerebral hemispheres, and displaying better sensitivity to treatment and prognosis than other gliomas. Oligodendroglioma constitutes 5–20% of all glial tumors and about 1500 new cases are diagnosed in Europe each year (Okamoto et al., 2007; Franco-Hernandez et al., 2009).

Recently, epigenetic alterations have also been identified in oligodendroglial tumors and primary include aberrant promoter hypermethylation of p16, p14, Rb1, p73, DAPKI and MGMT. Oligodendrogliomas frequently harbor the IDH1 mutation (up to 90%) and have a better prognosis as compared with other types of diffuse gliomas (Park et al., 2008; Klink et al., 2011). In uncontrolled analyses, approximately 50% of oligodendrogliomas in adults show LOH of chromosomes 1p and 19q. Interestingly, pediatric oligodendrogliomas do not appear to contain deletions of 1p or 19q as normal does. In low grade oligodendroglioma, LIH for 1p and 19q has been demonstrated in 38% of patients, and 5 year survival rates of 5% have been observed for those with deletions, serous 65% for those without deletions (Jaeckle et al., 2006).

In the present study, we investigated changes in protein expression in a 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 were extracted and evaluated by proteomic tools (2D-gel). Their alterations are

Abbreviations: 2-DG electrophoresis, two-dimensional gel electrophoresis; MS, mass spectrometry; MALDI, matrix assisted laser desorption ionization time-of-flight; WHO, World Health Organization; CNS, central nervous system.

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http://dx.doi.org/10.1016/j.gene.2015.05.039
0378-1119/

monitored using statistical data and specific software. The identification of differentially expressed proteins ($p < 0.05$ and fold > 2) was established by using MALDI TOF/TOF mass spectrometry.

2. Materials and methods

2.1. 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 at the Shohada Tajrish Hospital in Tehran, Iran. 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. Non-tumorous brain tissues were obtained from normal areas (either gray or white matter) of brain tissues removed from a patient undergoing non-tumor epileptic surgery. Eight tumor surgery operated patients with malignant oligodendroglioma have been separated, from which four tumors were selected for protein extraction and two-dimensional electrophoresis.

2.2. Tissue and sample preparation

Tissue samples of both tumoral and normal brains were snap-frozen immediately after operation in liquid nitrogen and stored at −80 °C until use for proteomic analysis. To obtain tissue extracts, the samples were broken into suitable pieces and were homogenized in lysis buffer II consisting of lysis buffer I {7 M urea, 2 M 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 on ice. Cell lysis was completed by subsequent sonication (4 × 30 pulses). The samples were then centrifuged at 20,000 ×*g* at 4 °C for 30 min to remove insoluble debris. The supernatants were combined with acetone 100% 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 was incubated for 1 h 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 Bradford test and spectrophotometry method, and the protein extracts were then separated and used for 2D gel electrophoresis.

2.3. 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 min 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. The second-dimension SDS-PAGE was carried out using 12% PAGE. Following SDS-PAGE, the gels were stained using the Coomassie Blue method (overnight).

2.4. Image analysis

Analytical gels were scanned by a Densitometer GS-800 (BioRad) scanner at 600 dpi in tagged image file format (TIFF). Image MasterTM 2D platinum v6.0 software was 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 by Progenesis Same Spots software to identify spots differentially expressed between tumor and control samples based on difference that were defined as altered. The spots were carefully matched individually and only spots that showed a definite difference were defined as altered. Spots were detected by pH isoelectric, molecular weights, databanks and comparison with previous research.

2.5. Mass spectrometric analysis

Spots were manually cut from 2-DE gels, each gel plug was soaked in 100 μL of washing solution (50% MeOH, 50 mM NH_4HCO_3) to re-swell and was washed two more times in the same solution. The gel plugs were further washed twice in 75% CAN, before being completely dried. Then, the samples were re-hydrated by adding freshly prepared trypsin solution (0.5 μg modified porcin trypsin in 25 μL 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% CAN, 0.1% TFA and transferred in a solution to a fresh 96 well plate, where the samples were dried.

Tryptic peptides were resuspended in 3 μL of 50% CAN, 0.1% TFA. 0.3 μL of resuspended, and were mixed (while wet) with 0.3 μL of a 90% saturated μ-cyano-4-hydroxycinnamic acid (CHCA) in 50% CAN, 0.1% TFA. The identification of expressed proteins ($p < 0.05$ and fold > 2) was established by using MALDI TOF/TOF MS. The samples were analyzed by MALDI TOF/TOF.

3. Results

Between February 2013 and February 2014, 10 patients (6 men and 4 women), with newly diagnosed high grade oligodendroglioma were entered into the study, and mean age was 57 years (range 42 to 79 years). Patients' individual characteristics for oligodendroglioma (III) are shown in Table 1. Eight patients out of 10 were chosen for laboratory analysis.

Using 2D-gel electrophoresis proteomic analysis, we compared protein expression patterns between the identities of expressed proteins ($p < 0.05$ and fold > 2) that were established by using MALDI

Table 1
Patients individual characteristics for oligodendroglioma (III).

No.	Age	Sex	History surgery	Region(s) studied	Extent of surgery	Response to treatment	Differential diagnosis
Case 1	55	W	Yes	Left frontal	Partial	Stable disease	High grade oligodendroglioma
Case 2	42	M	Yes	Left frontal	Partial	Stable disease	High grade oligodendroglioma
Case 3	79	M	Yes	Left hemisphere	Partial	Stable disease	High grade oligodendroglioma
Case 4	43	W	Yes	Left frontal	Partial	Stable disease	High grade oligodendroglioma
Case 5	60	M	Yes	Left hemisphere	Complete	Stable disease	High grade oligodendroglioma
Case 6	51	M	Yes	Left frontal	Partial	Stable disease	High grade oligodendroglioma
Case 7	53	W	Yes	Left frontal	Partial	Stable disease	High grade oligodendroglioma
Case 8	43	W	Yes	Left frontal	Partial	Stable disease	High grade oligodendroglioma
Case 9	71	M	Yes	Left frontal	Complete	Stable disease	High grade oligodendroglioma
Case 10	66	M	Yes	Left frontal	Partial	Stable disease	High grade oligodendroglioma