Article published online: 2024-05-17

Research Article

# Identification of Population-Specific Novel Protein Biomarkers and Possible Therapeutic Targets in Gliomas by Proteomics Approach

S. Devanand Senthil Kumar[1] Anbazhagan Periyasamy[1]

[1] Institute of Neurosurgery, Madras Medical College, Chennai, India

Indian J Neurosurg

**Address for correspondence** S. Devanand Senthil Kumar, MCh, Flat 1 D, Celestine Citadel, Vellalar Street, Mogappair, Chennai 600037, India (e-mail: dsk2059@gmail.com).

## Abstract

**Objective** To analyze the differential proteomic profile of gliomas in patients from South India and to identify novel protein glioma biomarkers and possible therapeutic targets to tailor the treatment to individual patients.

**Material and Methods** We have prospectively analyzed the differential proteomic profile of 34 patients with glioma imaging characteristics and compared them with that of normal brain tissue. This research was conducted at the Institute of Neurosurgery, Madras Medical College, in technical collaboration with the Indian Institute of Technology, Madras, over 1 year.

**Statistical Analysis** Biological variate analysis (I-ANALYSIS OF VARIANCE (ANOVA)) was used, with *p*-value less than 0.05 being significant.

**Results** Twenty proteins (10 upregulated and 10 downregulated) were differentially expressed in tumor tissue. The expression of three pro-apoptotic proteins was downregulated and the expression of three anti-apoptotic proteins was upregulated with statistical significance. The cellular functions of the 20 differentially regulated proteins were subjected to pathway analysis revealing significant alterations in heme biosynthesis, deoxyribonucleic acid (DNA) replication, fibroblast growth factor (FGF) signaling, and epidermal growth factor (EGF0 receptor signaling in glioma.

**Conclusion** KRT18, PRS4, and EF1A2 are anti-apoptotic proteins and are significantly upregulated in gliomas. EARS2, COX5A, and LSM3 are pro-apoptotic proteins, and are significantly downregulated in gliomas. This subverts the apoptotic pathways resulting in prolonged cell survival. This study's statistically significant dysregulation of these six proteins was unique, suggesting that they might be considered population-specific biomarkers and possible therapeutic targets for patients from South India. Abnormalities of heme biosynthesis at the proteomic level were identified in this study, which has not been very well studied previously.

**Keywords**

- glioma
- proteomics
- protein biomarkers
- dysregulation of proteins
- therapeutic targets

## Introduction

Brain tumors are now more frequently diagnosed, coinciding with the introduction of modern, newer imaging technology. Brain tumors represent approximately 85% of central nervous system (CNS) tumors, with gliomas being the most common.

The age-adjusted global annual incidence of gliomas for females is 17.2 per 100,000, and for males, it is 15.8 per 100,000.[1] A glioma cancer survey registry in India has

**DOI** https://doi.org/10.1055/s-0044-1786983.
**ISSN** 2277-954X.



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revealed the incidence of gliomas (per 100,000 population) to be 5.8, 6.7, 3.5, 5.6, and 28.2% among males, and 6.3, 5.6, 7.5, 0, and 21.8% among females, in the cities of Mumbai, Bangalore, Chennai, Dibrugarh, and Trivandrum, respectively.[2]

Today's optimal care for gliomas, which includes gross total resection, radiation therapy, and concomitant or subsequent chemotherapy, has helped improve the median survival in low-grade gliomas from 44 months in 1999 to 57 months in 2010. The benefits have been even more modest in glioblastoma multiforme (GBM), with patients living for an average period of 15 months after diagnosis, despite the development of newer modalities of imaging, early identification of high-grade lesions, development of newer chemotherapy agents and technical advancements in radiation delivery.

This failure can be ascribed mainly to the one mutation–one drug formula we have followed so far. This tunnel vision—a strategy to block only one or two of the mutated or downregulated cancer signaling pathways—has led to the failure of various therapeutic agents.

Recently, many markers and characteristic signatures have been identified as factors with prognostic and predictive value in GBM using newer techniques of molecular and genetic profiling. Not one of them has been successful in clinical practice. The reasons are varied. Although the initiating and important step in the expression of genes is transcription, resulting in the formation of messenger RNA (mRNA), the stability of mRNA and the resultant protein determines the protein levels.[3] Alternate splicing of a gene results in the formation of multiple proteins. So, three or more proteins can be encoded by a single human gene. Proteins undergo modifications after translation, which regulate their functions by altering their interactions, turnover, and location. Proteins, as the functional units of cells, influence the genesis of cancer by varying their synthesis rate, levels, and interactions. Defective signaling pathways involving an entire network of proteins result in cancer development and progression.[3] Moreover, the huge costs involved in setting up and managing the infrastructure and logistics for genomic labs, and the issues of their availability and accessibility preclude their routine use in the management of gliomas in this part of the world.

Therefore, there is an overwhelming need to develop newer molecular diagnostic tools to identify and develop more potential protein biomarkers from biopsies, serum, or plasma[4] for the early detection of tumor growth, recurrence, and therapeutic response, and also for the delivery of personalized, individualized oncologic cure through precision medicine. The methods of protein extraction and quantification are relatively more economical and more widely available when compared to genetic labs.

## Glioma Proteomics

Proteomics deals with the analysis of proteins produced by a cell, tissue, or organism at a specific time and in a specified environment. Differentiating the proteomic profile of brain tumors vis-a-vis normal brain tissue enables one to detect and monitor the pathological changes by the discovery of potential protein biomarkers for the diagnosis, prognosis, prediction, or assessment of response to treatment of CNS neoplasia, particularly gliomas. This also helps develop possible therapeutic targets in the population under study.

Proteomic analysis can be done using glioma biopsy specimens, cerebrospinal fluid (CSF) and tumor cyst fluid, plasma/serum, cell lines of glioma, and animal models.[5] The latter sources, though easy to collect, have several limitations like low biomarker concentrations, biomarker dilution in the peripheral blood, the presence of the blood–brain barrier, and the presence, in plasma, of proteins from the whole body (not from the CNS alone). These factors restrict their use in the discovery of biomarkers.[6]

So, tumor tissue analysis provides a direct approach to discovering glioma-specific protein biomarkers.[6] Even tissue sampling has its difficulties and limitations that preclude its routine use as a tool for clinical diagnosis. These include its invasive nature, the inadequate volume of tissue obtained to perform statistical analysis, and inherent sampling errors.

These limitations could be circumvented by other methods of sampling, and proteomics permits analysis with lesser quantity of tissue. The understanding of tumor biology and tumor characterization is enhanced by tissue-derived biomarkers.[5]

Most studies for glioma biomarker discovery have been done using tumor specimens. Studies conducted in 2004[7,8] identified many grade-specific biomarkers that were dysregulated in astrocytoma specimens when compared to normal tissue. The use of brain tumor specimens and nontumoral brain tissue to identify differential protein expression patterns was demonstrated by Schwartz et al.[7] Using gel-based and mass spectrometry-based techniques, many studies have reported such differential protein profiling involving αB-crystallin, glial fibrillary acidic protein (GFAP), and many other proteins including Ki-67.[9,10] Newer proteins like TIMP-1 (tissue inhibitor of metalloproteinases 1), neurofascin, and UCHL-1 (ubiquitin carboxy-terminal hydrolase-1) with possible grade specificity, and the known glioma markers—AnxA2 and calnexin—have been the subject of further functional assay verification.[11]

Eighty-five tissue specimens (52 glioblastoma, 13 anaplastic astrocytoma, 10 diffuse astrocytoma, and 10 normal brain samples) were subjected to proteomic analysis by Iwadate et al, and 57 protein spots with potential for differentiating tumors from the normal brain were identified.[12] Immunohistochemical analysis confirmed that grade IV tumors exhibited increased expression of four proteins—VREB1, GRP78, RhoA, and Rac1—and decreased expression of enolase when compared to lower-grade tumors. Odreman et al, in 2005, analyzed 10 grade II and 10 grade IV specimens and identified 15 proteins that showed differential expression in these two grades.[13] The proteins

Peroxiredoxin 1 and 6, the transcription factor BTF3, and R-B-crystallin were more highly expressed in GBM, as against GFAP, protein disulfide isomerase A3, and the cAMP-dependent protein kinase's catalytic subunit, which showed higher expression in low-grade astrocytomas. Using 2 dimensional electrophoresis (2DE) and Matrix Assisted Laser Desorption Ionization- Time of Flight (MALDI-TOF) spectrometry, Hiratsuka et al found 11 proteins to be upregulated and 4 to be downregulated in glioma.[14] Glioma downregulation of SIRT2 was confirmed by Northern blotting. High levels of SIRT2 caused malfunction of the microtubule network and prevented colony stabilization, demonstrating its possible tumor suppressor function. Ngo et al endeavored to show that glioma cells that differed in their chromosome 1p status expressed proteins differently.[15] They revealed that decreased expression of stathmin, a microtubule-associated protein, was related to loss of heterozygosity and longer recurrence-free survival in anaplastic oligodendroglioma patients. Patients with $1p^{+/-}$ anaplastic oligodendroglioma tumors with less stathmin expression had improved outcomes on treatment with nitrosourea, possibly due to an increased mitotic arrest in cells caused by decreased levels of stathmin. This was also confirmed in mice carrying xenograft tumors, suggesting an inverse correlation between overall survival and stathmin levels.

Other proteins could also alter the sensitivity of gliomas to chemotherapy. Galectin 1 has high expression in glioma cell lines. Puchades et al, using control U87 glioma cells or p53 adenovirus-infected cells treated with SN38, found that downregulation of galectin1 by p53 was further enhanced by SN38 treatment, suggesting that galectin 1 could be targeted for therapy.[10]

But all the studies mentioned earlier have been conducted with patients from developed Western countries.

In our study, using tumor biopsy specimen and employing recent proteomic techniques, we have endeavored to investigate the differential proteomic profile for glioma in South Indian patients as compared to normal tissue and, in the process, identify novel glioma protein biomarkers and possible therapeutic targets to tailor the treatment to individual patients.

## Material and Methods

We have prospectively analyzed the differential proteomic profile of 34 patients with glioma imaging characteristics and compared them with that of normal brain tissue. This research was conducted at the Institute of Neurosurgery, Madras Medical College, in technical collaboration with the Indian Institute of Technology, Madras, over 1 year.

### Inclusion Criteria

Patients with imaging characteristics of glioma admitted to the Institute of Neurosurgery, Madras Medical College, and Rajiv Gandhi Government General Hospital and who consented to participate in the study.

### Exclusion Criteria

- Patients not consenting for surgery.
- Patients not consenting to take part in the study.

Forty patients with glioma imaging characteristics who consented to be involved in this study were included. Six patients were excluded from the study due to insufficient RNA extraction from the tumor sample, leaving 34 samples for proteomic analysis. The patients' demographic data, relevant past medical and family history, imaging characteristics, and operative details were recorded.

The tumor tissue specimen was bisected, and one-half was used for histopathological examination. The other half of the tumor was taken for proteomic analysis. In liquid nitrogen, the portion for proteomic analysis was snap frozen and stored at –80°C within 15 minutes of surgical resection and then transported to the lab. Non-neoplastic matched reference samples were obtained from patients with severe diffuse axonal injury who underwent decompressive craniectomy. The tissues were deposited at the National Cancer Tissue Biobank, Department of Biotechnology, Indian Institute of Technology, Madras, where proteomic analysis was performed using 2DE and tandem mass spectrometry (►**Fig. 1**). Proteins that were dysregulated (up- or downregulated) in tumor tissue when compared to normal tissue were identified. A mixture of identical amounts of all the samples to be analyzed was used as an internal control, and this allowed accurate measurement of differential protein expression, irrespective of the number of matched gels, and random matching of normal tissue and tumor samples in each gel.

The statistical significance of the dysregulation status was analyzed with biological variate analysis with $p$-value less than 0.05 considered statistically significant.

## Results

The protein expression data were analyzed in 34 patients. There were 22 males and 12 females in the study group. The mean age of the patients was 39.6 years, and the range was from 14 to 65 years.

The patients were classified as grades I to IV based on the WHO grading. There were 2 patients in grade I, 5 in grade II, 6 in grade III, and 21 in grade IV.[16]

Twenty proteins (10 upregulated and 10 downregulated) were found to be expressed differentially in tumor samples in comparison to normal brain tissue in the same gels, as shown in ►**Figs. 2**–**4**.

With reference to the available database for glioma, correlation analysis of 20 differentially expressed target proteins revealed that upregulation of 3 (KRT18, PRS4, and EF1A2) of the 10 upregulated proteins and downregulation of 3 (EARS2, COX5A, and LSM3) of the 10 downregulated proteins were statistically significant (►**Table 1**).

The cellular functions for the 20 differentially regulated proteins were subjected to pathway analysis revealing significant alterations in heme biosynthesis (25%), DNA

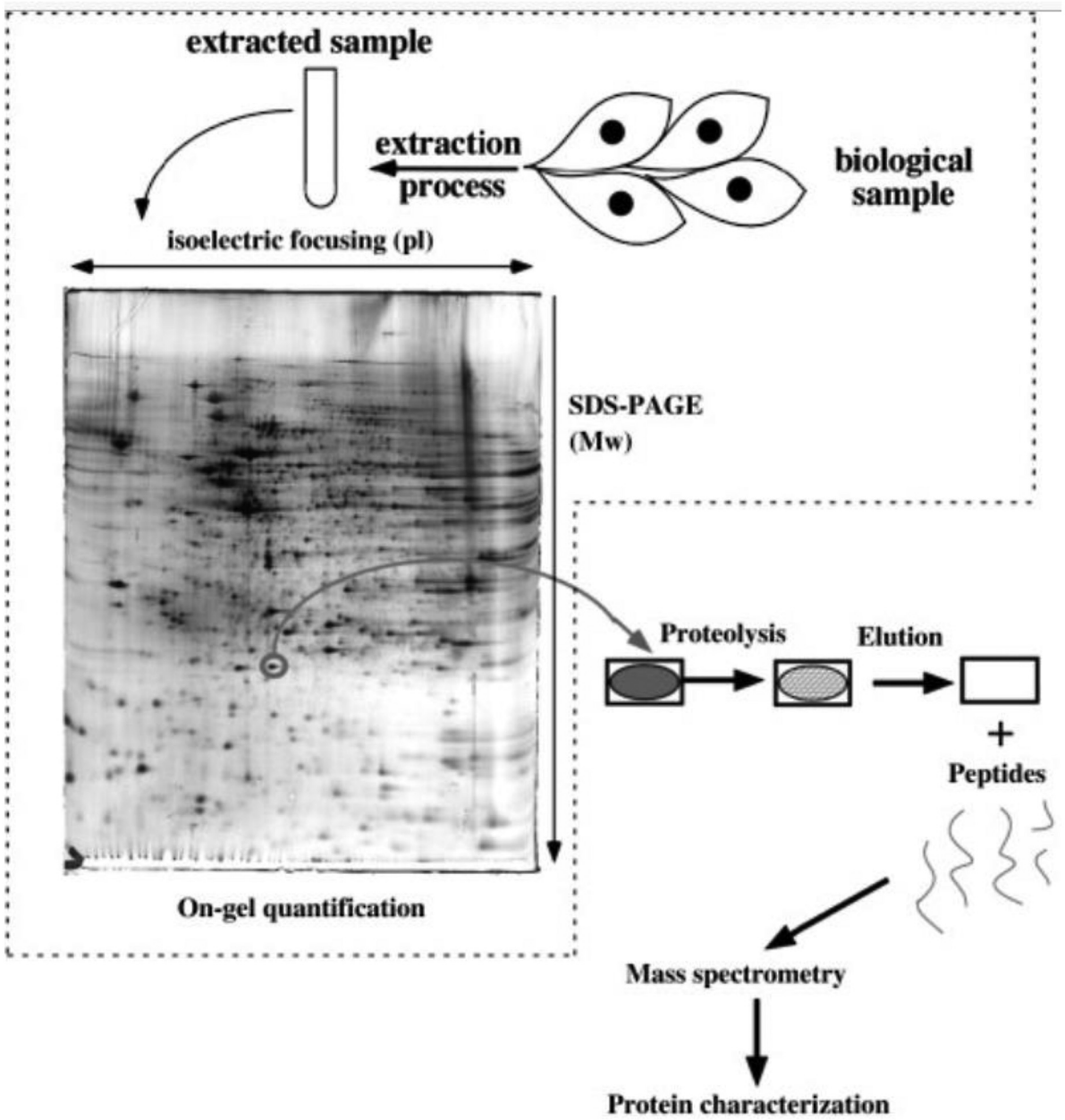



**Fig. 1** Extraction of sample and identification of proteins.

replication (25%), FGF signaling (25%), and EGF receptor signaling (25%) in glioma.

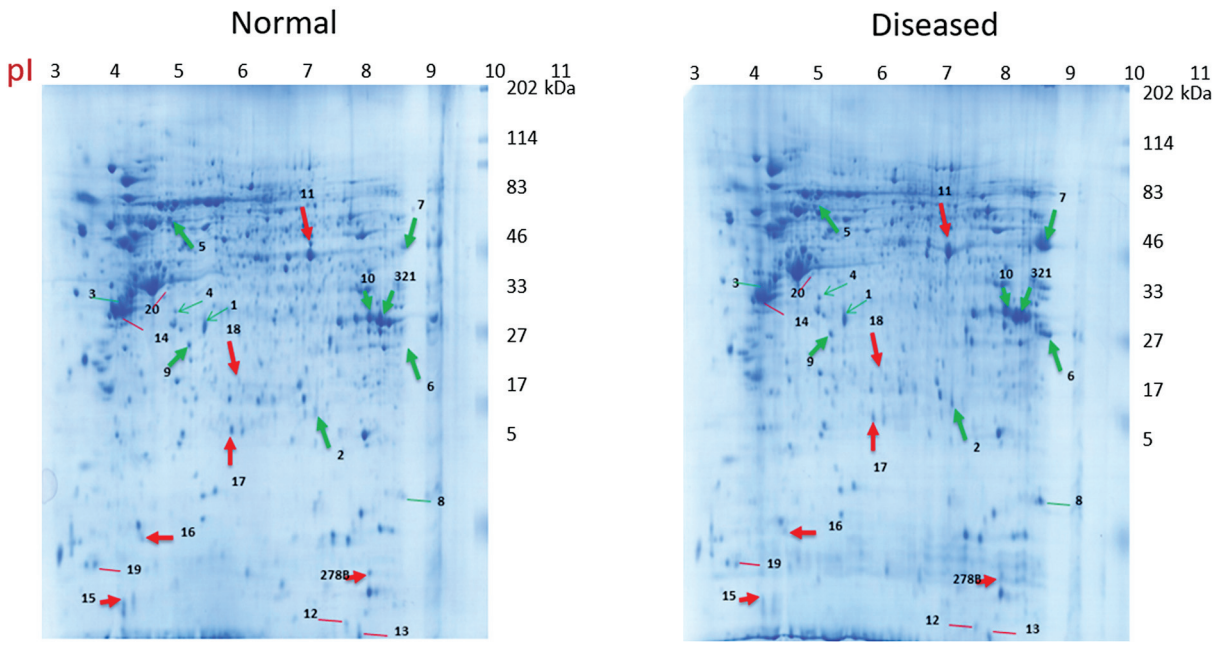
## Discussion

Our study used 2DE coupled with tandem mass spectrometry for proteomic analysis of glioma tumor samples and to identify differentially expressed protein signatures in glioma tumor samples. We identified 20 dysregulated proteins in glioma, which were validated by comparison with normal tissue specimens in the same gels.

Among 20 dysregulated proteins identified, 10 proteins, including phosphoglycerate phosphatase (PGP), Ras-related protein Rab-5C (RAB5C), KRT18, annexin A3 (ANXA3), 26S protease regulatory subunit 4 (PRS4), nitric oxide synthase interacting protein (NOSIP), elongation factor 1-alpha 2 (EF1A2), NADH dehydrogenase iron-sulfur protein 4 (NDUS4), serine/threonine protein phosphatase 6 (PPP6), and aminoacyl tRNA synthase complex interacting multifunctional protein-1 (AIMP1), were upregulated in glioma.

In contrast, 10 proteins including notchless protein homolog 1 (NLE1), hemoglobin alpha-1 globin chain (HBA1), probable glutamate tRNA ligase (EARS2), proliferating cell nuclear antigen (PCNA), cytochrome-c oxidase subunit 5A (COX5A), uncharacterized protein (H7BZT4), GrpE protein homolog 1 (GRPEL1), pyridoxine 5′-phosphate oxidase variant (Q53FP0), LSM3, an arginase-2 (ARGI2), were downregulated in glioma in comparison to normal brain tissue samples.




| Up regulated | Down regulated |
|---|---|
| 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 | 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 |

**Fig. 2** Separation of proteins using two-dimensional (2D) electrophoresis. *Green arrows* indicate upregulated proteins and *red arrows* indicate downregulated proteins. *More prominent spots or darker spots* signify increased expression (upregulation), and *smaller or lighter spots* signify decreased expression (downregulation). For example, the spot for protein number 7 appears smaller and lighter in normal tissue when compared to the tumor tissue, implying upregulation in tumor tissue.

With reference to the available database for glioma, correlation analysis of the 20 differentially expressed target proteins revealed that upregulation of 3 (KRT18, PRS4, and EF1A2) of the 10 upregulated proteins and downregulation of 3 (EARS2, COX5A, and LSM3) of the 10 downregulated proteins were statistically significant (►**Table 1**), suggesting that these 6 proteins might be considered as population-specific biomarkers for the South Indian population because they have not been found to be statistically significantly dysregulated in any other study (►**Fig. 5**). These analyses highlight the importance of biomarker-based population screening and their potential to be used in future therapeutic strategies for better glioma management.

These results suggest that among the 20 dysregulated proteins modulating cell survival cascade, the statistically significant downregulation of pro-apoptotic proteins—EARS2, COX5A, and LSM3—and upregulation of anti-apoptotic proteins—KRT18, PRS4, and EF1A2—in glioma samples in comparison to matched normal tissue signify their action of promoting cell survival during tumorigenesis. Evasion of apoptosis, being a significant hallmark of cancer, can be used for targeted treatment to prevent tumor progression.[17] Reduced expression of pro-apoptotic proteins and elevated expression of anti-apoptotic proteins result in prolonged cell survival by circumvention of the apoptotic pathways.

The cellular functions for the 20 differentially regulated proteins were subjected to pathway analysis, revealing significant alterations in heme biosynthesis, DNA replication, FGF signaling, and EGF receptor signaling in glioma. Although abnormalities of DNA regulation, EGFR signaling pathway, and FGF signaling pathway have been studied in detail previously, abnormalities of heme biosynthesis have not been so very well studied.

## Conclusion

- This study has identified 20 dysregulated proteins modulating cell survival cascade in human brain gliomas. Out of these, six proteins (KRT18, PRS4, EF1A2, EARS2, COX5A, and LSM3) have not been previously reported as being statistically significantly dysregulated in brain gliomas, probably because they are unique to the test cohort, namely, South Indian population.
- KRT18, PRS4, and EF1A2 are anti-apoptotic and are significantly upregulated in gliomas.
- EARS2, COX5A, and LSM3 are pro-apoptotic and are significantly downregulated in gliomas.
- Abnormalities of heme biosynthesis have not been so very well studied previously. To our knowledge, this is the first

Up regulated

| Spot ID | Accession | Description | Score | Coverage | MW [kDa] | calc. pI |
|---|---|---|---|---|---|---|
| 1 | A6NDG6 | Phosphoglycolate phosphatase OS=Homo sapiens GN=PGP PE=1 SV=1 - [PGP_HUMAN] | 34.89 | 41.12 | 34 | 6.14 |
| 2 | P51148 | Ras-related protein Rab-5C OS=Homo sapiens GN=RAB5C PE=1 SV=2 - [RAB5C_HUMAN] | 36.83 | 59.72 | 23.5 | 8.41 |
| 3 | I6L965 | KRT18 protein (Fragment) OS=Homo sapiens GN=KRT18 PE=2 SV=1 - [I6L965_HUMAN] | 72.74 | 56.72 | 42.1 | 5.1 |
| 4 | P12429 | Annexin A3 OS=Homo sapiens GN=ANXA3 PE=1 SV=3 - [ANXA3_HUMAN] | 41.07 | 29.5 | 36.4 | 5.92 |
| 5 | P62191 | 26S protease regulatory subunit 4 OS=Homo sapiens GN=PSMC1 PE=1 SV=1 - [PRS4_HUMAN] | 96.38 | 54.55 | 49.2 | 6.21 |
| 6 | Q9Y314 | Nitric oxide synthase-interacting protein OS=Homo sapiens GN=NOSIP PE=1 SV=1 - [NOSIP_HUMAN] | 54.55 | 46.18 | 33.2 | 8.82 |
| 7 | Q05639 | Elongation factor 1-alpha 2 OS=Homo sapiens GN=EEF1A2 PE=1 SV=1 - [EF1A2_HUMAN] | 485.3 | 58.1 | 50.4 | 9.03 |
| 8 | O43181 | NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitochondrial OS=Homo sapiens GN=NDUFS4 PE=1 SV=1 - [NDUS4_HUMAN] | 51.35 | 61.14 | 20.1 | 10.3 |
| 9 | O00743 | Serine/threonine-protein phosphatase 6 catalytic subunit OS=Homo sapiens GN=PPP6C PE=1 SV=1 - [PPP6_HUMAN] | 25.61 | 20.33 | 35.1 | 5.69 |
| 10 | Q12904 | Aminoacyl tRNA synthase complex-interacting multifunctional protein 1 OS=Homo sapiens GN=AIMP1 PE=1 SV=2 - [AIMP1_HUMAN] | 44.04 | 55.77 | 34.3 | 8.43 |

**Fig. 3** Spot ID, accession number, protein name, score, coverage %, number of covering peptides, molecular weight (calc MW), and calculated isoelectric point za (calc. pI) of the upregulated proteins.

study to discover heme biosynthesis abnormalities at the proteomics level.
- The usefulness of population screening for biomarkers and its potential use in future therapeutic strategies for enhanced and individualized glioma management is highlighted by this study.

## Limitations of the Study

- Grade I gliomas that show different biological behavior could have been left out from the study.
- Grade-specific proteomic profiling for glioma grades II to IV was not performed in our study due to lesser number of samples of lower-grade gliomas. This would have improved the prognostication value of the study.
- The proteomic profile of the participants of our study was compared with that of the Western population. It would have been more relevant to have used participants from other parts of India for comparison.
- A larger cohort of participants would improve the statistical significance of the findings.

## Future Directions

- Grade-specific proteomic profiling of gliomas will be a more useful diagnostic and prognosticating tool.
- Region-specific proteomic profiling would help better understand the differences in the biological behavior of these tumors.
- Many newer proteins dysregulated in glioma are being validated as brain cancer biomarkers through immunoblotting and immunohistochemical analysis. If they are successfully validated, their functional and biological characterization may allow them to be used as diagnostic and prognostic markers, as therapeutic targets, to predict responses to therapy, and also to identify new molecular targets for therapy. Further studies with larger cohorts are required to achieve this.

### Ethical Approval Statement

The approval was obtained from the Institutional ethics committee.

Down regulated

| Spot ID | Accession | Description | Score | Coverage | MW [kDa] | calc. pI |
|---|---|---|---|---|---|---|
| 11 | Q9NVX2 | Notchless protein homolog 1 OS=Homo sapiens GN=NLE1 PE=1 SV=4 - [NLE1_HUMAN] | 44.57 | 40.62 | 53.3 | 7.34 |
| 12 | E9M4D4 | Hemoglobin alpha-1 globin chain (Fragment) OS=Homo sapiens GN=HBA1 PE=3 SV=1 - [E9M4D4_HUMAN] | 70.37 | 49 | 10.8 | 8.48 |
| 13 | I3L166 | Probable glutamate--tRNA ligase, mitochondrial OS=Homo sapiens GN=EARS2 PE=2 SV=1 - [I3L166_HUMAN] | 125.6 | 18.75 | 12.4 | 10.42 |
| 14 | P12004 | Proliferating cell nuclear antigen OS=Homo sapiens GN=PCNA PE=1 SV=1 - [PCNA_HUMAN] | 50.71 | 38.7 | 34.8 | 4.69 |
| 15 | P20674 | Cytochrome c oxidase subunit 5A, mitochondrial OS=Homo sapiens GN=COX5A PE=1 SV=2 - [COX5A_HUMAN] | 587.71 | 61.33 | 16.8 | 6.79 |
| 16 | H7BZT4 | Uncharacterized protein OS=Homo sapiens PE=4 SV=1 - [H7BZT4_HUMAN] | 241.71 | 34.74 | 10.8 | 5.5 |
| 17 | Q9HAV7 | GrpE protein homolog 1, mitochondrial OS=Homo sapiens GN=GRPEL1 PE=1 SV=2 - [GRPE1_HUMAN] | 88.46 | 65.44 | 24.3 | 8.12 |
| 18 | Q53FP0 | Pyridoxine 5'-phosphate oxidase variant (Fragment) OS=Homo sapiens PE=2 SV=1 - [Q53FP0_HUMAN] | 53.41 | 45.98 | 29.9 | 7.4 |
| 19 | B2R5H5 | cDNA, FLJ92476, highly similar to Homo sapiens LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM3), mRNA OS=Homo sapiens PE=2 SV=1 - [B2R5H5_HUMAN] | 149.4 | 66.67 | 11.8 | 4.7 |
| 20 | P78540 | Arginase-2, mitochondrial OS=Homo sapiens GN=ARG2 PE=1 SV=1 - [ARGI2_HUMAN] | 58.83 | 57.06 | 38.6 | 6.47 |

**Fig. 4** Spot ID, accession number, protein name, score, coverage %, number of covering peptides, molecular weight (calc MW), and calculated isoelectric point za (calc. pI) of downregulated proteins.

**Table 1** Twenty differentially regulated proteins with name, statistical significance value, and regulation status obtained from the biological variate analysis

| Sl. no. | Name | I-ANOVA/*p*-value | Regulation in tumor |
|---|---|---|---|
| 1. | PGP | 0.703 | Up |
| 2. | RAB5C | 0.482 | Up |
| **3.** | **KRT18** | **0.012** | **Up** |
| 4. | ANXA3 | 0.135 | Up |
| **5.** | **PRS4** | **0.027** | **Up** |
| 6. | NOSIP | 0.452 | Up |
| **7.** | **EF1A2** | **0.043** | **Up** |
| 8. | NDUS4 | 0.624 | Up |
| 9. | PPP6 | 0.675 | Up |
| 10. | AIMP1 | 0.171 | Up |
| 11. | NLE1 | 0.256 | Down |
| 12. | HBA1 | 0.335 | Down |
| **13.** | **EARS2** | **0.05** | **Down** |
| 14. | PCNA | 0.685 | Down |
| **15.** | **COX5A** | **0.0056** | **Down** |

(*Continued*)

**Table 1** (*Continued*)

| Sl. no. | Name | I-ANOVA/*p*-value | Regulation in tumor |
|---|---|---|---|
| 16. | H7BZT4 | 0.189 | Down |
| 17. | GRPE1 | 0.815 | Down |
| 18. | Q53FPO | 0.230 | Down |
| **19.** | **LSM3** | **0.00096** | **Down** |
| 20. | ARGI2 | 0.464 | Down |

Note: The proteins shown in bold fonts are statistically significantly dysregulated.

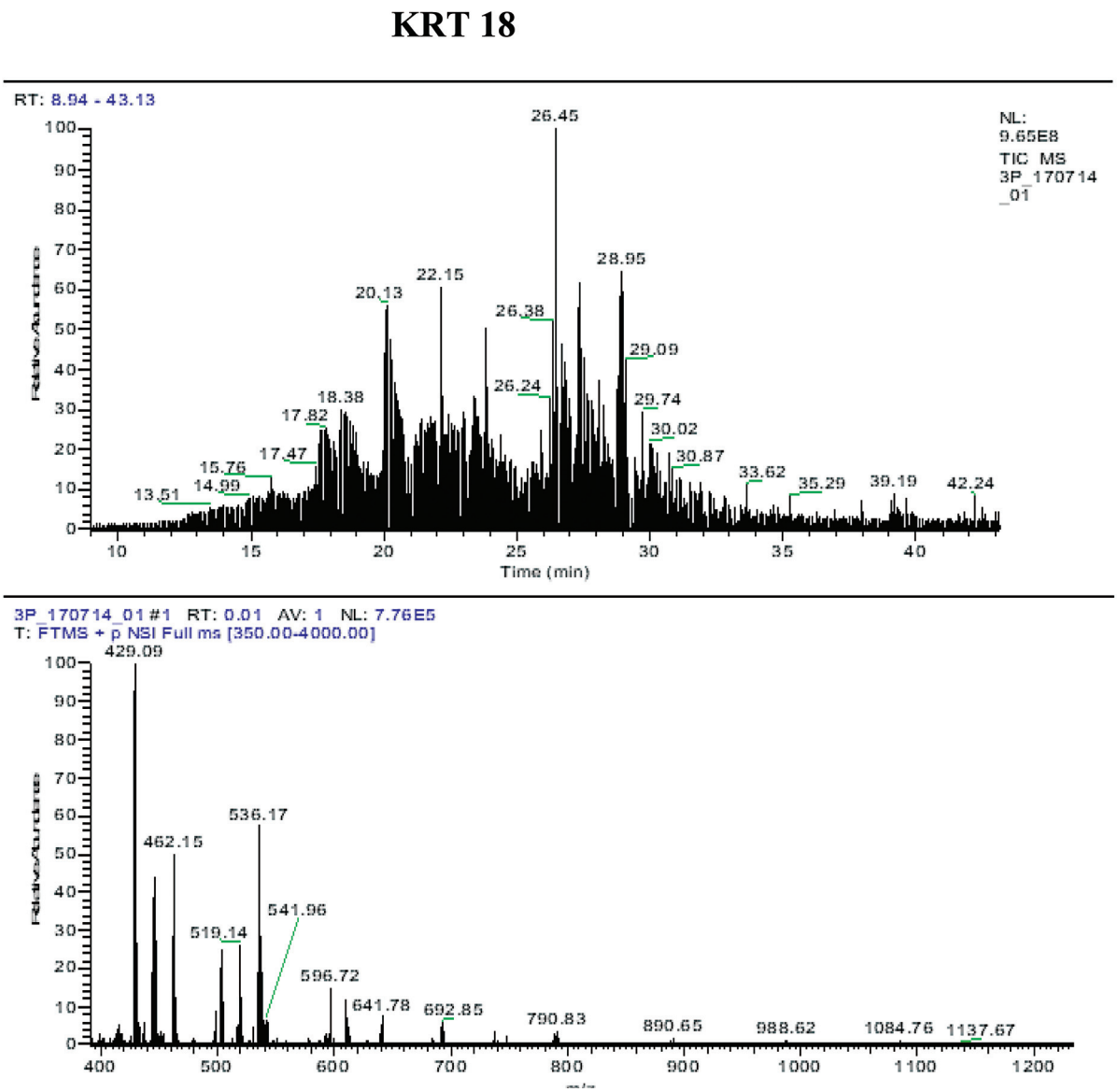



**Fig. 5** Spot identification of protein KRT 18 showing good spectrum (as an example).

**Conflict of Interest**
None declared.

## References

1 Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. Acta Neuropathol 2005;109(01):93–108
2 Nandakumar A, Ramnath T. Consolidated report of Hospital-based registries 2004–2006. National Cancer Registry Programme. Bangalore: Indian Council for Medical Research; 2009:54
3 Somasundaram K, Nijaguna MB, Mohankumar D. Serum proteomics of glioma: methods and applications. Expert Rev Mol Diagn 2009;9(07):695–707

4 Cervi D, Yip TT, Bhattacharya N, et al. Platelet-associated PF-4 as a biomarker of early tumor growth. Blood 2008;111(03):1201–1207
5 Niclou SP, Fack F, Rajcevic U. Glioma proteomics: status and perspectives. J Proteomics 2010;73(10):1823–1838
6 Furuta M, Weil RJ, Vortmeyer AO, et al. Protein patterns and proteins that identify subtypes of glioblastoma multiforme. Oncogene 2004;23(40):6806–6814
7 Schwartz SA, Weil RJ, Johnson MD, Toms SA, Caprioli RM. Protein profiling in brain tumors using mass spectrometry: feasibility of a new technique for the analysis of protein expression. Clin Cancer Res 2004;10(03):981–987
8 Chumbalkar VC, Subhashini C, Dhople VM, et al. Differential protein expression in human gliomas and molecular insights. Proteomics 2005;5(04):1167–1177
9 Park CK, Jung JH, Park SH, Jung HW, Cho BK. Multifarious proteomic signatures and regional heterogeneity in glioblastomas. J Neurooncol 2009;94(01):31–39
10 Puchades M, Nilsson CL, Emmett MR, et al. Proteomic investigation of glioblastoma cell lines treated with wild-type p53 and cytotoxic chemotherapy demonstrates an association between galectin-1 and p53 expression. J Proteome Res 2007;6(02):869–875
11 Karas M, Hillenkamp F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem 1988;60(20):2299–2301
12 Iwadate Y, Sakaida T, Hiwasa T, et al. Molecular classification and survival prediction in human gliomas based on proteome analysis. Cancer Res 2004;64(07):2496–2501
13 Odreman F, Vindigni M, Gonzales ML, et al. Proteomic studies on low- and high-grade human brain astrocytomas. J Proteome Res 2005;4(03):698–708
14 Hiratsuka M, Inoue T, Toda T, et al. Proteomics-based identification of differentially expressed genes in human gliomas: down-regulation of SIRT2 gene. Biochem Biophys Res Commun 2003; 309(03):558–566
15 Ngo TT, Peng T, Liang XJ, et al. The 1p-encoded protein stathmin and resistance of malignant gliomas to nitrosoureas. J Natl Cancer Inst 2007;99(08):639–652
16 Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, eds. WHO Classification of Tumours of the Central Nervous System. Lyon: IARC; 2007
17 Horne SD, Pollick SA, Heng HHQ. Evolutionary mechanism unifies the hallmarks of cancer. Int J Cancer 2015;136(09):2012–2021