The proteomic response in glioblastoma in young patients

Citation for published version:
Deighton, RF, Le Bihan, T, Martin, SF, Barrios-Llerena, ME, Gerth, AMJ, Kerr, LE, McCulloch, J & Whittle, IR 2014, 'The proteomic response in glioblastoma in young patients', Journal of Neuro-Oncology, vol. 119, no. 1, pp. 79-89. https://doi.org/10.1007/s11060-014-1474-6

Digital Object Identifier (DOI):
10.1007/s11060-014-1474-6

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Journal of Neuro-Oncology

Publisher Rights Statement:
This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
The proteomic response in glioblastoma in young patients

Ruth F. Deighton · Thierry Le Bihan · Sarah F. Martin · Martin E. Barrios-Llerena · Alice M. J. Gerth · Lorraine E. Kerr · James McCulloch · Ian R. Whittle

Abstract Increasing age is an important prognostic variable in glioblastoma (GBM). We have defined the proteomic response in GBM samples from 7 young patients (mean age 36 years) compared to peritumoural-control samples from 10 young patients (mean age 32 years). 2-Dimensional-gel-electrophoresis, image analysis, and protein identification (LC/MS) were performed. 68 proteins were significantly altered in young GBM samples with 29 proteins upregulated and 39 proteins downregulated. Over 50 proteins are described as altered in GBM for the first time. In a parallel analysis in old GBM (mean age 67 years), an excellent correlation could be demonstrated between the proteomic profile in young GBM and that in old GBM patients ($r^2 = 0.95$) with only 5 proteins altered significantly ($p < 0.01$). The proteomic response in young GBM patients highlighted alterations in protein–protein interactions in the immunoproteosome, NFkB signalling, and mitochondrial function and the same systems participated in the responses in old GBM patients.

Keywords Clinical proteomics · Glioblastoma · Patient age

Introduction

Patients diagnosed with Glioblastoma (GBM, WHO-IV) have extremely poor median survival times, despite modern microsurgery, chemoradiotherapy, reoperation and experimental therapies [1–4]. To improve GBM treatment and patient median survival times, fresh insight into the molecular pathogenesis of GBM is essential. Proteomics can define molecular pathways and cellular functions altered in GBM [5]. Genomic studies, although important, are limited by the fact that normal, upregulated or mutated genes may not be transcribed for a number of epigenetic reasons [6]. Multiple discrepancies between mRNA and proteomic expression profiles in differential analyses of gliomas highlight the importance of studying protein expression [7].

Age is a powerful individual prognostic indicator [8–10]. Long term survivors of GBM are invariably younger patients [8–10], and in one randomised clinical trial, median survival for GBM cohorts aged <45 years was 48 weeks compared to 19 weeks for those >65 years; and at 18 months 23 % of the younger cohort was alive compared to 3 % of the older cohort [8]. Numerous randomised controlled trials and hospital series have excluded differences in access to health care as the cause for this differential outcome [1, 9–12].

The biological basis of this powerful age-related effect is not understood. The histological features of GBM,
cellular proliferative indices, epidermal growth factor receptor amplification and p53 expression are very similar irrespective of age [9]. Although younger patients are more likely to have secondary GBM than the elderly [3, 9, 13], there is no difference in outcomes between primary and secondary GBM once the diagnosis is made and patients have been aged matched with controls [13]. Although genetic differences associated with short survival (6q loss, 10q loss, 19q gain, sodium ion channel mutations) and longer survival (TP53 mutations and the combination of LOH1p and LOH19q, MGMT status, mutation of IDH1) have been identified, these differences have not, with the exception of IDH1 mutations where the mutation occurred in younger patients, been analysed with respect to patient age [14–20].

Analyses of GBM samples from older patients has begun to provide a coherent view of the proteomic response in GBM but interpretation is complicated by differences in experimental design and proteomic technology [5]. In this study we provide the first systematic proteomic analysis in young GBM (versus age-matched peritumoural-control brain) to gain insight into the basis of the importance of age on prognosis. For the purpose of comparison, we performed a parallel, contemporaneous study (using the same experimental design and technology) in old GBM.

Materials and methods

Clinical material

Glioblastoma and peritumoural-control brain samples were obtained from young (<45 years) and old patients (>60 years) undergoing resective brain tumour surgery (Ethical approval: LREC/2004/4/16). The sampling procedure and clinical details of experimental samples are described in Supplementary Tables 1 and 2 and Supplementary-methods. The experimental group sizes used for the primary proteomic analysis were young GBM (n = 7) and young peritumoural-control (n = 12) (based on a priori power calculations to detect significant changes of ≥35% with power ≥0.8). Tissue was collected for two comparison groups: old GBM (n = 13) and old peritumoural-control (n = 10). The median coefficients of variation were similar in each experimental group: young GBM 33.55%, young peritumoural-control 27.38%, old GBM 33.99% and old peritumoural-control 26.39% (Supplementary Fig. 1).

Total protein extracts were separated by isoelectric point and molecular mass using 2DGE (see Supplementary-methods). 2D-gel images were captured using a FluorChem Image Analyser and aligned in a single study using at least four manual alignment vectors followed by automatic placement of further alignment vectors by the software (~200–400 vectors per gel). The mean protein levels of each protein were analysed using Student’s t test (p ≤ 0.003, equivalent to p ≤ 0.01 with Bonferroni correction factor 3 for each comparison). Significant data are presented in Table 1 (uncorrected for multiple comparisons) and all data are presented in Supplementary Table 3. Protein spots differentially expressed in young GBM versus young peritumoural-control were manually excised and proteins identified using LC–MS [21]. LC–MS runs of each sample were combined using Maxquant, assuming a false positive rate of 0.01 [22]. An identical approach was applied in parallel comparing old GBM versus old peritumoural-control, and young GBM versus old GBM.

Immunoblot analysis was performed on a subset of the tissue samples used for proteomics. Proteins (10 μg) were separated by SDS-PAGE and transferred to nitrocellulose membrane. Primary antibodies were detected using fluorescently-labelled secondary antibodies and were visualized using an Odyssey Imager.

To assess functional protein–protein interactions between the proteins altered in young GBM (p ≤ 0.01), altered protein identifiers were uploaded to Ingenuity Pathway Analysis (IPA; http://www.ingenuity.com). Networks were algorithmically generated based on direct relationships (physical interactions and/or associations) between eligible proteins. Networks are scored and ranked according to the inclusion of as many proteins inputted as possible. Network scores are putatively a measure of probability [23]. For comparison purposes, network analysis was also performed on proteins differentially regulated in old GBM versus old peritumoural-control. Network analysis is a powerful tool for identifying potential interactions between altered proteins (hypothesis generation) which can be subsequently explored in functional analyses.

Results

The median survival in the young GBM cohort was >39 months (3 of the 7 patients are still alive) and this was significantly greater than the median survival of 9 months in the older reference group (p < 0.02). Performance status (see Supplementary Table 1) was not significantly different between the young (mean age 36 years) and old (mean age 67 years) GBM cohorts.
Table 1 Proteins altered in young GBM

| Spot ID | Protein ID | Protein accession number | Young GBM Fold change | p value | Old GBM Fold change | p value | Main protein function |
|---------|------------|--------------------------|-----------------------|---------|--------------------|---------|-----------------------|
| 746     | CKMT1A     | P12532                   | 0.32                  | 1.76E-08 | 0.53               | 3.95E-05 | ATP homeostasis       |
| 749     | GNB1*      | P62873                   | 0.59                  | 2.05E-07 | 0.63               | 2.29E-05 | GPCR beta subunit     |
| 757     | DYSPLS2    | Q16555                   | 0.4                   | 3.94E-07 | –                  | –       | Cytoskeletal          |
| 798     | INA        | Q16352                   | 0.36                  | 4.21E-07 | 0.59               | 0.00240 | Cytoskeletal          |
| 271     | ALDOA      | P04075                   | 0.71                  | 5.48E-07 | 1.3                | 0.00034 | Glycolysis            |
| 310     | CRYM       | Q14894                   | 0.37                  | 7.79E-07 | 0.55               | 9.96E-05 | –                     |
| 768     | STMN1      | P16949                   | 0.38                  | 8.37E-07 | 0.59               | 0.00015 | Cytoskeletal          |
| 161     | GDI2       | P50395                   | 0.34                  | 9.28E-07 | –                  | –       | –                     |
| 119     | OXCT1      | P55809                   | 0.37                  | 9.71E-07 | –                  | –       | Lipid metabolism      |
| 67      | DYSPLS2    | Q16555                   | 0.45                  | 1.11E-06 | 0.63               | 0.00246 | –                     |
| 760     | VDAC1C     | P45880                   | 0.53                  | 1.28E-06 | 0.53               | 1.40E-06 | Ion transport         |
| 763     | GOT1       | P17174                   | 0.53                  | 2.09E-06 | 0.53               | 1.86E-05 | Amino acid metabolism |
| 736     | GNB1*      | P62873                   | 0.43                  | 2.32E-06 | 0.47               | 1.05E-06 | GPCR beta subunit     |
| 343     | NAPB       | Q9H115                   | 0.5                   | 2.34E-06 | 0.55               | 7.05E-06 | Ca²⁺ mediated exocytosis |
| 469     | NDFUS3     | Q75489                   | 2.9                   | 2.83E-06 | 2.5                | 3.00E-06 | Electron transport    |
| 492     | C1orf128   | Q9GZP4                   | 0.36                  | 3.55E-06 | 0.5                 | 2.36E-05 | unknown               |
| 120     | OXCT1      | P55809                   | 0.48                  | 3.70E-06 | 0.63               | 0.00026 | Lipid metabolism      |
| 451     | PNPO*      | B4E152                   | 2.1                   | 6.70E-06 | –                  | –       | Pyridoxine biosynthesis |
| 249     | TUBB2A     | Q13885                   | 0.5                   | 7.44E-06 | –                  | –       | Cytoskeletal          |
| 243     | –          | –                        | 0.59                  | 1.25E-05 | 0.59               | 1.29E-05 | –                     |
| 938     | MBP        | P02686                   | 0.53                  | 1.36E-05 | –                  | –       | Myelin                |
| 809     | PSAT1      | Q9Y617                   | 0.53                  | 1.45E-05 | –                  | –       | Amino acid biosynthesis |
| 84      | INA        | Q16352                   | 0.33                  | 1.51E-05 | 0.45               | 3.21E-05 | Cytoskeletal          |
| 613     | PGAM1      | P18669                   | 0.33                  | 1.68E-05 | 0.55               | 0.00044 | Glycolysis            |
| 428     | PSM21      | Q06323                   | 2.3                   | 1.76E-05 | 2.0                | 3.40E-05 | Immunoproteosome      |
| 734     | TUBB2A     | Q13885                   | 0.42                  | 1.79E-05 | 0.48               | 1.46E-06 | Cytoskeletal          |
| 785     | UCHL1      | P09936                   | 0.59                  | 1.89E-05 | 0.55               | 9.13E-06 | Stabilises free ubiquitin |
| 544     | TAGLN3     | Q9U115                   | 0.5                   | 2.55E-05 | 0.5                 | 4.52E-07 | Neuronal growth       |
| 1046    | TUBB2C     | P68371                   | 0.45                  | 2.59E-05 | 0.59               | 0.00113 | Cytoskeletal          |
| 774     | PDXP       | Q96GD0                   | 0.33                  | 2.62E-05 | 0.5                 | 4.72E-05 | Phosphatase activity  |
| 794     | PRDX3*     | P30048                   | 1.9                   | 2.72E-05 | –                  | –       | Antioxidant           |
| 823     | HPR1       | P00492                   | 1.6                   | 3.32E-05 | –                  | –       | Purine synthesis      |
| 379     | VDAC2      | P45880                   | 0.71                  | 3.67E-05 | –                  | –       | Ion transport         |
| 748     | NAPG       | Q99747                   | 0.45                  | 4.70E-05 | 0.59               | 9.04E-06 | Vesicle transport     |
| 459     | UCHL1      | P09936                   | 0.77                  | 4.77E-05 | –                  | –       | Stabilises free ubiquitin |
| 657     | UBE2 N     | P61088                   | 2.2                   | 5.20E-05 | –                  | –       | Ubiquitination        |
| 786     | SEPT11     | Q92599                   | 0.59                  | 6.54E-05 | 0.63               | 0.00129 | Vesicle transport     |
| 828     | PRDX3      | P30048                   | 0.66                  | 7.37E-05 | 0.77               | 0.00058 | Antioxidant           |
| 812     | PSME2      | Q9UL46                   | 2.0                   | 9.5E-05  | 2.0                | 0.00169 | Immunoproteosome      |
| 822     | HSPD1      | P10809                   | 0.63                  | 9.5E-05  | 0.63               | 0.00173 | Chaperone             |
| 1062    | HSPB1      | P04792                   | 0.5                   | 0.000106 | 0.71               | 0.00219 | Chaperone             |
| 69      | DYSPLS2    | Q16555                   | 0.66                  | 0.000118 | –                  | –       | Cytoskeletal          |
| 285     | ACOT7      | O00154                   | 0.5                   | 0.000124 | 0.53               | 2.84E-05 | Acetyl-CoA binding    |
| 605     | MBP*       | P02686                   | 0.53                  | 0.000131 | –                  | –       | Myelin                |
| 116     | PHGDH      | O43175                   | 0.55                  | 0.000132 | 0.53               | 0.00187 | Serine biosynthesis   |
| 467     | GFAP       | P14136                   | 2.4                   | 0.000148 | –                  | –       | Cytoskeletal          |
| 483     | DCXR*      | Q7Z4W1                   | 2.7                   | 0.000186 | –                  | –       | Glucose metabolism    |
Table 1 continued

| Spot ID | Protein ID | Protein accession number | Young GBM | Old GBM | Main protein function |
|---------|------------|--------------------------|-----------|---------|-----------------------|
|         |            | Fold change | p value | Fold change | p value |
| 868     | UCHL1      | P09936      | 0.66     | 0.000229 | –         | Stabilises free ubiquitin |
| 276     | IDH3A      | P50213      | 0.48     | 0.000239 | 0.59      | 3.65E−06 TCA cycle |
| 916     | CKB        | P12277      | 0.66     | 0.000245 | 0.66      | 1.49E−05 ATP homeostasis |
| 487     | TPI        | D3DUS9      | 2.1      | 0.000251 | –         | Glycolysis |
| 556     | PEBP1      | P30086      | 2.7      | 0.000255 | –         | Intracellular signaling |
| 718     | DCD        | A5HP3       | 0.37     | 0.000259 | 0.43      | 3.06E−06 Phosphatase activity |
| 66      | DPYSL2     | Q16555      | 0.63     | 0.000316 | –         | Cytoskeletal |
| 92      | CCT6A      | P40227      | 2.2      | 0.000391 | 2.0       | 0.000454 Protein folding |
| 91      | HIST1H4A*  | P62805      | 1.6      | 0.000479 | –         | Chromatin binding |
| 498     | GRB2       | P62993      | 0.63     | 0.000499 | –         | Signal transduction |
| 273     | hCG_2002*  | Q59GE1      | 0.71     | 0.000548 | 0.66      | 6.60E−05 Neuron growth |
| 579     | DCD        | A5HP3       | 2.4      | 0.000596 | –         | Phosphatase activity |
| 62      | DPYSL2     | Q16555      | 1.7      | 0.000689 | –         | Cytoskeletal |
| 756     | ATP6V1E1   | P36543      | 0.71     | 0.000709 | 0.63      | 2.82E−05 Energy metabolism |
| 437     | CLIC*      | Q9Y696      | 2.4      | 0.000737 | 2.5       | 0.000155 Ion transport |
| 288     | TUBB2A     | Q13885      | 0.66     | 0.000745 | –         | Cytoskeletal |
| 731     | TF*        | P02787      | 1.5      | 0.000749 | –         | Iron transfer |
| 843     | PDIA3      | P30101      | 1.4      | 0.000751 | 1.3       | 0.00263 Protein folding |
| 409     | HSPA5      | P11012      | 2.0      | 0.000810 | 1.8       | 0.00035 Chaperone |
| 488     | APOA1*     | P02647      | 0.55     | 0.000811 | 0.53      | 0.00026 Lipid metabolism |
| 270     | ALDOA      | P04075      | 0.71     | 0.000941 | 0.66      | 0.00124 Glycolysis |
| 771     | GFAP*      | P14136      | 1.8      | 0.000971 | 1.7       | 0.000953 Cytoskeletal |
| 740     | HSPB1      | P04792      | 0.66     | 0.00101  | 0.71      | 0.00193 Chaperone |
| 898     | GLUD1*     | P00367      | 1.4      | 0.00107  | –         | Glutamate turnover |
| 966     | ATP6V1B2   | P21281      | 0.59     | 0.00112  | –         | Energy metabolism |
| 263     | OvBr SEPT  | Q9UHD8      | 0.66     | 0.00114  | –         | Cytoskeletal |
| 789     | hCG_2002   | Q59GE1      | 0.59     | 0.00115  | –         | Neuronal growth |
| 801     | SEPT11     | Q9NVA2      | 0.63     | 0.00124  | 0.66      | 0.00104 Vesicle transport |
| 154     | SEPT11     | Q9NVA2      | 0.71     | 0.00133  | 0.63      | 0.00161 Vesicle transport |
| 28      | GDP2       | P43304      | 1.9      | 0.00149  | –         | Lipid metabolism |
| 207     | ACTR1B     | P42025      | 0.63     | 0.00169  | –         | Cytoskeletal |
| 25      | HSPA8      | P11142      | 0.63     | 0.00187  | –         | Chaperone |
| 1012    | SRI        | P30626      | 1.8      | 0.00191  | –         | Calcium homeostasis |
| 516     | GSTP1      | P09211      | 1.3      | 0.00192  | 1.2       | 0.000867 Free radical clearance |
| 342     | DKFZp686   | P07355      | 1.4      | 0.00193  | 1.9       | 0.00220 unknown |
| 572     | TAGLN3     | Q9U115      | 2.8      | 0.00200  | –         | Neuronal growth |
| 81      | CAT        | P04040      | 1.4      | 0.00218  | –         | Nucleotide binding |
| 434     | GSTO1      | P78417      | 1.5      | 0.00234  | –         | Glutathione metabolism |
| 444     | ACOT7      | O00154      | 1.6      | 0.00239  | –         | Acetyl-CoA binding |
| 838     | PGAM1      | P18669      | 1.6      | 0.00249  | –         | Glycolysis |
| 876     | ALAD*      | P13716      | 1.6      | 0.00251  | –         | Haeme production |
| 324     | TUBB2B     | Q9BVA1      | 0.77     | 0.00263  | –         | Cytoskeletal |
| 403     | GNB1       | P62873      | 0.63     | 0.00269  | –         | GPCR subunit |
| 829     | SNCG       | A9XXE1      | –        | –        | 0.38      | 7.58E−09 unknown |
| 945     | HIST1H4A   | P62805      | –        | –        | 1.6       | 1.79E−05 Chromatin binding |
| 277     | ALDOA      | P04075      | –        | –        | 0.71      | 3.98E−05 Glycolysis |
| 401     | CLIC1      | O00299      | –        | –        | 0.55      | 0.000135 Ion transport |
Young GBM: proteins differentially expressed in young GBM compared to age matched controls

A total of 405 protein spots were matched across every 2D gel (young GBM and young peritumoural-control gels) and analysed. Logarithmic association of the 405 protein expression levels (mean normalised volumes) highlights multiple protein alterations in young GBM (Fig. 1a, Supplementary Fig. 2). 90 protein spots were altered in young GBM (versus young peritumoural-control; \( p \leq 0.01 \)) and the identity of these 90 statistically significant altered spots was established by LC-MS. Sixty eight unique proteins were significantly altered in young GBM (Table 1, Supplementary Table 3). 15 of these proteins were identified multiple times in 2–5 spots (ATP6V1B2, OXCT1, ALDOA, GFAP, DCD, DPYSL2, TUBB2A, INA, MBP, ACOT7, VDAC2, UCHL1, PGAM1, PRDX3 and GNB1). Identification of the same protein in several spots is a feature of 2DGE proteomic studies and explains the difference between the number of altered protein spots and number of unique proteins identified. From the 68 altered proteins identified, 29 proteins were up-regulated and 39 proteins were down-regulated. A major fraction of the proteins altered in young GBM (25 %; 16 out of the 68 proteins) are putatively localised to mitochondria (OXCT1, PEBP1, DPYSL2, CKMT1A, ACOT7, CKB, IDH3A, SNAP, VDAC2, PRDX3, HSPD1, CAT, ATP6V1E1, GLUD1, CLIC4 and NDUFS3). 12 of the 68 proteins altered in young GBM have previously been described altered in proteomic studies of glioma (APOA1, GFAP, HSPA5, PDIA3, TUBB2A, GLUD1, GSTP1, PGAM1, UCHL1, HSPB1, HSPD1 and SRI) [5]. Notably, over 50 proteins have been described altered in GBM for the first time.

Ten proteins (DPYSL2, SRI, OXCT1, UCHL1, CAT, SEPT11, IDH3A, PDIA3, ATP6V1B2, PRDX3), altered in young GBM were examined using western blotting. Western blotting of young GBM versus young peritumoural-control tissue, demonstrated that 7 out of the 10 proteins tested were significantly altered (\( p \leq 0.01 \)) and that 10 out of the 10 proteins showed the same direction of response as the proteomic analysis (Fig. 2, Supplementary Fig. 3).

### Table 1 continued

| Spot ID | Protein ID | Protein accession number | Young GBM | Old GBM | Main protein function |
|---------|------------|--------------------------|-----------|---------|----------------------|
|         |            |                          | Fold change | \( p \) value | Fold change | \( p \) value |
| 1073    | UQCRFSL    | P0C7P4                   | –          | –       | 0.66      | 0.000261           | unknown               |
| 772     | PSMB7      | Q99436                   | –          | –       | 1.5       | 0.000261           | 20 s proteosome       |
| 564     | PEBP1      | P30086                   | –          | –       | 0.71      | 0.000298           | Intracellular signaling |
| 840     | MAP2K1     | Q02750                   | –          | –       | 0.71      | 0.000313           | Intracellular signaling |
| 317     | LASP1      | Q14847                   | –          | –       | 1.8       | 0.000322           | Cytoskeletal           |
| 466     | –          | –                        | –          | –       | 1.7       | 0.000437           | –                     |
| 217     | GLUL       | P15104                   | –          | –       | 0.55      | 0.000471           | Glutamine synthesis   |
| 223     | SUCLA2     | Q9P2K7                   | –          | –       | 0.55      | 0.000493           | TCA cycle             |
| 299     | DDAH1      | Q94760                   | –          | –       | 0.71      | 0.000548           | NO regulation         |
| 227     | CKB        | P12277                   | –          | –       | 0.77      | 0.000996           | ATP homeostasis        |
| 601     | SOD1       | P00441                   | –          | –       | 0.63      | 0.00102            | Antioxidant           |
| 419     | PFAH1B2    | P68402                   | –          | –       | 1.5       | 0.00111            | –                     |
| 1028    | –          | –                        | –          | –       | 1.5       | 0.00129            | –                     |
| 443     | –          | –                        | –          | –       | 1.9       | 0.00185            | –                     |
| 530     | PRDX1      | Q06830                   | –          | –       | 1.5       | 0.00214            | Antioxidant           |
| 653     | PRDX5      | P30044                   | –          | –       | 0.77      | 0.00237            | Antioxidant           |
| 845     | GLUL       | P15104                   | –          | –       | 0.63      | 0.00238            | Glutamine synthesis   |
| 375     | –          | –                        | –          | –       | 0.71      | 0.00257            | –                     |

Proteins significantly altered in young GBM relative to young peritumoural controls are listed (ordered by \( p \) value). Only significant protein changes are listed (\( p \) values shown are prior to Bonferroni correction with a factor 3). Spot ID provides a unique 2DGE spot identifier and is important because several proteins were identified in multiple spots, for example OXCT1 in spot 119 and spot 120. Proteins marked with an asterisk indicate a spot where a second protein (or occasionally more) is present at a level close to that of the listed protein. Blank protein IDs (for example spot 243) represent where protein identity could not be established. The protein accession numbers (Uniprot), magnitude of protein response and \( p \) values (ranked according to changes in young GBM) are listed for each altered protein. For comparison, proteins significantly altered in old GBM, relative to old controls are listed. Blank values, for example Spot 757 (DPYSL2) in old GBM, indicate that the significant change in this protein in young GBM did not achieve statistical significance in the old cohort (see Supplementary Table 3 for more details and information on fold change and probability levels for proteins that failed to reach the pre-determined significance level (i.e. \( p < 0.003 \)).
IPA network analysis was performed on the proteomic dataset referred to as young GBM and included 68 proteins. The young GBM dataset generated multiple functional protein networks (Table 2) including 4 high scoring networks containing 23, 15, 12 and 11 dataset proteins respectively (Table 2).

The top network generated by IPA (Fig. 3) included multiple structural proteins downregulated in young GBM, for example Strathmin (STMN1) and dihydropyrimidinase-related protein 2 (DPYS2). The network also contained GFAP, upregulated in young GBM, which has long been considered a fundamental and diagnostic feature of glioma [23]. The network included heat shock proteins (HSPD1, HSPA8, HSPB1), and a group of downregulated proteins involved in ATP homeostasis and energy metabolism (ALDOA, ATP6V1E1, CKB, CKMT1A), consistent with existing evidence but also identifying for the first time specific protein networks that may be involved in the dysregulation of energy metabolism in malignant glioma [24]. Lastly a cluster of upregulated proteins, integral to the immunoproteosome (PSME1, PSME2, 20 s/26 s proteosome, PSMBT7), was highlighted in the top network. Network 2 was characterised by a cluster of Septin proteins (GTPase proteins that have been shown to play a role in gliomagenesis [25], and the insertion of a hub protein, TRAF6, a signal transducer in NFkappaB signalling. Network 3 was characterised by the insertion of a hub protein HNF4alpha, a transcription factor recently shown to play a role in other neoplasias [26] and Network 4 was characterised by numerous mitochondrial-localised proteins (CAT, IDH3A, NDUFS3 and other complex 1 proteins, OXCT1 and PRDX3).

Old GBM: proteins differentially expressed in Old GBM compared to age matched control tissue

To allow the extensive protein alterations in young GBM to be compared with those in old GBM, a proteomic evaluation was conducted contemporaneously in old GBM (patients >60 years) using the same technology. A total of 405 protein spots were matched across every 2D gel (old GBM and old peritumoural-control gels) and analysed. Logarithmic association of the 405 protein expression levels (mean normalised volumes) was broadly similar to that seen in young GBM (Supplementary Fig. 2). 70 protein spots were altered in old GBM versus old peritumoural-control (p < 0.01).

55 unique proteins were altered significantly in old GBM (listed in Table 1 and Supplementary Table 3 in full). 8 of these proteins were identified multiple times in 2-4 spots (GBN1, INA, ALDOA, SEPT11, HSPB1, CKB, CLIC and GLUL). From the 55 altered proteins identified, 16 proteins were up-regulated and 39 proteins were down-regulated. 19 of the 55 proteins have been reported to be putatively localised to mitochondria.
Proteomic response in young GBM: a comparison with the proteomic response in old GBM

Five protein spots were differentially altered between young and old GBM (p < 0.01). Only three unique statistically altered proteins were identified (PEBP1, NDUFA10 and PGK1). The proteins in two spots were not identified. This number of altered proteins lies beneath the multiple testing threshold of potential false positive results in the study. There was an excellent correlation (r² = 0.95) between the level of 405 proteins analysed in GBM from younger patients and their level in GBM from older patients (Fig. 1b). This correlation was similar to that seen in peritumoural-control samples from the two age groups (Supplementary Fig. 2). There were good correlations (r² = 0.85 and 0.90) between the level of 405 proteins in young GBM relative to young peritumoural-controls (Fig. 1a) and old GBM relative to old peritumoural-control (Supplementary Fig. 2) respectively. 48 unique proteins...
Table 2  Putative interactions between proteins altered in young GBM and old GBM

| Network ID | Molecules in YOUNG Networks | Score | Focus Molecules | Network ID | Molecules in OLD Networks | Score | Focus Molecules |
|------------|-----------------------------|-------|-----------------|------------|---------------------------|-------|-----------------|
| 1          | 26s proteasome, ALDOA, alpha tubulin, ANXA2, ATP6V1E1, beta tubulin, CCT6A, CKB, CLIC4, creatine kinase, CRMP2-KLC1-tubulin, DNAJ12, DNAJ6, DPYSL2, DPYSL5, GARP, GAPA1, GRB2, GSTP1, HSP, HSPA8, HSPB1, HSPD1, INA, IL1R1, ITGB3, MAP2K1, PDIA3, PSME1, PSME2, STMN1, TF, TUBB2A, TUBB2C, Tubulin, TUBE2N | 53    | 23              | 1          | 26s proteasome, ALDOA, alpha tubulin, ANXA2, Ap1, ATP6V1E1, beta tubulin, CCT6A, CKB, CLIC4, creatine kinase, CRMP2-KLC1-tubulin, DPYSL2, DPYSL5, GARP, GAPA1, GSTP1, Hsp90, HSPD1, INA, MAP2K1, PDIA3, PSMB1, PRDX1, PSMB7, PSME1, PSME2, SNCG, STMN1, TUBB2A, TUBB2C, Tubulin | 54    | 23              |
| 2          | ACTR1B, ALAD, ARHGEF18, CDC42EP3, Cdk1, CRMP1, DPCR, GD2, GH1, GLU1D1, GNB1, GDPD2, HPRT1, HSPA4, KRT33, KRT33B, MAP4K3, PEBP1, PHGDH, Rab11A, Rab4C, SEPT2, SEPT4, SEPT5, SEPT6, SEPT7, SEPT8, SEPT9, SEPT11, SEPT14, Septin5, SH2D2A, Traf6, YWHAG | 31    | 15              | 2          | AL52, APOA1, APOA4, APOC1, APOC2, ARRB2, CDC42EP3, CLIC1, DCD, GNB1, GOT1, HNF4A, HSP, IDH1A, LASP1, MAP3K1, NDUFS3, NDUFS4, NDUFS7, OXCT1, PAFH1B2, PAR2K, PCMT1, PGAM1, PHGDH, PNP, PON1, PPARG1A, PPRDX5, SAA, SH2D2A, SOD1, Traf6, TUBB2C, UCHL1 | 35    | 16              |
| 3          | ACOT7, APOA1, APOA4, APOC1, BMP2K, CDC42EP3, CLYBL, DCD, DHR52, DLG4, F11, Gapdh, GOT1, GPD1, GSTD1, GSTZ1, HNF1A, HNF4A, HSP, ITIH4, IFR, NAP1, NAP6, NSF, palmitoyl-CoA hydroxylase, PEX13, PNPO, PON1, PSAT1, SAA, Snare, SR1, UCHL1, VDAC1, VDAC2 | 23    | 12              | 3          | ACOT7, ARHGEF18, Caveolin, DLG4, GNB5, GRIA2, HERCS, HSPB1, HSPD1, HSPH1, IGF2, MAPKAPKS, MYC, NAP1, NAP6, NSF, PRDX1, PRDX2, PRDX3, RPL1, SEPT2, SEPT4, SEPT5, SEPT7, SEPT8, SEPT9, SEPT11, Septin, SercinC3, Snare, SNRPD, STXB1, SUC2, TOMM40, VDAC2 | 24    | 12              |
| 4          | ALDOA, ATP5B, ATP5O, ATPV1B2, CAT, CFCL1, Gollin, HSPD1, IDH3A, Mapk, MT-COII, MT-ND1, MT-ND2, MT-NDS, MT-ND4, MT-ND5, NDUFA6, NDUFA9, NDUFB6, NDUFB7, NDUFB8, NDUFB9, OXCT1, PABPN1, PDXP, PGAM1, PPARG1A, PPRDX1, SNCA, TPP1, YWHAE | 21    | 11              | 4          | CDC73, PITHD1 | 2     | 1               |
| 5          | CDC73, PITHD1 | 2     | 1               | 5          | DDAH1, N1F1, RPS6KA1 | 2     | 1               |

Protein–protein interaction networks were generated by IPA (http://www.ingenuity.com). The proteins highlighted in bold are the proteins found significantly altered in the study (t test \( p \leq 0.01 \), with Bonferroni correction factor 3) in young GBM (relative to young peritumoural control) and old GBM (relative to old control), and are termed ‘Focus Molecules’. Proteins not in bold have been inserted by IPA and are proteins that interact with the focus molecules. The coloured arrows indicate the direction of response of the focus molecules in GBM (red = upregulated; green = downregulated). Each network is assigned a score by IPA. Network scores are putatively a measure of probability for the network (but see [23] for critical analysis of this issue).

The young and old networks display many common features. For example, Network 1 (the highest scoring network) in young GBM contains 23 focus molecules and 17 of these (ALDOA, ANXA2, ATP6V1E1, CCT6A, CKB, CLIC4, DPYSL2, GARP, GSTP1, HSPD1, INA, PDIA3, PSME1, PSME2, STMN1, TUBB2A, TUBB2C) are also found in Network 1 in old GBM.
altered in GBM were common in young and old cohorts. The direction and fold change of all 48 proteins was consistent in both young and old GBM. From the top 25 altered protein spots identified in Young GBM (ranked by p value), 17 were demonstrated altered in old GBM (p < 0.01).

Discussion

The present study provides a powerful example of how proteomics can reliably test a hypothesis (i.e. is the most important prognostic variable in GBM, age, associated with a distinct response) and demonstrates that proteomics can play an important role in understanding GBM pathophysiology. Definition of the proteomic response in samples from patients with a homogeneous and clinically defined age range (18–45 years) addresses one of the design weaknesses in proteomic studies of GBM to date [5].

Young (<45 years of age) and old (>60 years of age) GBM cohorts with a mean age difference of 31 years and a significantly better median survival despite optimal therapy in the younger cohort were recruited. Multiple protein alterations were detected in young and old GBM versus age matched control tissue, and included a mixture of previously well-characterised protein alterations in GBM (for example, GFAP and UCHL1), and the identification of many ‘highly expected’ heat shock proteins (HSPD1, HSPB1, HSPA5, HSPA8) and cytoskeletal proteins (TUBB2A, TUBB2C), which confirm the robustness of our proteomic data.

One cluster of upregulated proteins (Fig. 3) in both young and old GBM comprised PSME1, PSME2, 20 s/26 s proteosome and PSMB7. These interacting proteins are central to the immunoproteosome (i-proteosome). All proteasomes contain a 20 s subunit flanked by either 19 s subunits or 11 s subunits. In the standard proteasome two 19 s subunits enclose a 20 s subunit of 2α rings sandwiching 2β rings with proteolytic subunits (β1, β2, β5). In the i-proteasome these catalytic subunits are substituted by LMP2, MECL, LMP7 and the 20 s is flanked by two 11 s/PA28 subunits. The 11 s contains 3α & 3β alternating subunits regulated by PSME1 and PSME2 respectively [27], two of the proteins upregulated in our young and old GBM analyses. Inhibition of the 20 s/26 s proteasome by
drugs, such as carfilzomib leads to a build up of poly-ubiquinated proteins causing cell cycle arrest, apoptosis and inhibition of tumour growth [28].

i-proteasome function is to provide peptides for MHC-class1-antigen presentation. Interferon increases i-proteasome numbers during inflammation and oxidative damage [29]. 26 s proteasomes are ineffective at degrading oxidised proteins, but i-proteasomes can efficiently process these damaged proteins [27]. Increased PSME1 and PSME2 as a result of interferon, would prevent protein build up and apoptosis. Conversely loss of i-proteasome function, through inhibition of 11 s subunit formation or joining of the 11 s subunit to the 20 s, would have the two fold effect of damaged protein aggregation, leading to apoptosis; and the removal of ‘self’ peptides from the cell surface, alerting the immune system to the malignant tumour cells. Elucidating the mechanisms of GBM immune resistance and causes of immunosuppression is currently an area of intense research and therapeutic effort in GBM [30, 31].

The proteomic analyses of young and old GBM also highlighted multiple proteins (PRDX3, UCHL1, PEBP1, DPYSL2, UBE2 N, GSTO) involved in nuclear factor kappaB (NFkB) regulation. NFkB is a transcription factor capable of mediating many cellular responses and modulates oncogenesis, tumour progression and chemotherapy resistance [32–35]. In the cytoplasm, NFkB is a small protein complex containing two subunits that bind inhibitory kappa B (IκB). IκB binding prevents NFκB translocation to the nucleus. Activation of NFκB, with subsequent translocation to the nucleus can occur through the canonical (utilising IκB kinase, IKK), the non-canonical or the alternative pathway. In the nucleus NFκB regulates transcription of proteins that down-regulate apoptosis, increase cell invasiveness, increase angiogenesis and increase vascular permeability, thereby promoting tumourogenesis [32, 36]. Proteins that regulate NFκB function were altered in GBM. GSTO1 (upregulated in the young GBM analysis) increases IL1β levels which activates IKK. GSTO1 also increases Akt phosphorylation in cells exposed to the pro-apoptotic drug cisplatin. Phosphorylated Akt inhibits apoptosis via NFκB [37]. UBE2 N (also upregulated in the young GBM analysis) is also vital for the activation of IKK via TRAF6 [38]. TRAF6, a core signal transducer in the NFκB pathway was highlighted as a hub protein in IPA Network 2 of both young and old GBM IPA analyses. PRDX3 (downregulated in young and old GBM analyses) also increases IKK activation [39], and knock-down studies of UCHL1 (also downregulated in young and old GBM analyses), show an increase in NFκB function via IKK activation [40]. PEBP1 (upregulated in young GBM and downregulated in old GBM) antagonises NFκB function by interfering with the TNFα pathway [41], resulting in an increase in NFκB function. NFκB’s role in gliomagenesis is summarised in Supplementary Fig. 5. NFκB inhibitors have shown promise in inducing cell death in GBM [42].

Alignment of protein alterations identified in young and old GBM versus age-matched peritumoural-controls showed considerable commonality in the proteomic response of GBM in different aged patients (and also demonstrated the rigour of our two distinct proteomic analyses of GBM). Our study does not provide a clear explanation as to why young and old patients with GBM have differential prognoses. One of the few proteins putatively altered in expression level between young and old GBM, is Phosphatidyl ethanolamine binding protein 1 (PEBP1; also known as Raf1-kinase inhibitor protein, RKIP). PEBP1 was found significantly upregulated in our young GBM proteomic analysis, significantly downregulated in our old GBM analysis, and significantly downregulated in old GBM compared to young GBM. PEBP1 inhibits the RAF/MEK/ERK pro-oncogenic pathway and also inhibits NFκB (also pro-oncogenic) by antagonising the activity of IKK either directly or via Tumour Necrosis Factor alpha (TNFalpha) [41]. The difference in PEBP1 expression levels between young and old GBM could contribute to their different prognosis.

Acknowledgments This work was supported by grants from the Chief Scientist Office, The Melville Trust, and The Brain Tumour Research Fund. RFD is funded by The Melville Trust as their research fellow. TLB, SFM, MEBL, and LEK are funded by SynthSys Edinburgh which is a Centre for Integrative Systems Biology (CISB) funded by BBSRC and EPSRC; reference BB/D019621/1.

Conflict of interest The authors report no conflicts of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Anderson E, Grant R, Lewis SC, Whittle IR (2008) Randomized phase III controlled trials of therapy in malignant glioma: Where are we after 40 years? Brit J Neurosurg 22:339–349
2. Davis FG, Freels S, Grutsch J, Barlas S, Brem S (1998) Survival rates in patients with primary malignant brain tumours stratified by patient age and tumor histological type: an analysis based in surveillance, epidemiology, and end results (SEER) data, 1973–1991. J Neurosurg 88:1–10
3. Preusser M, de Ribaupeire S, Wöhrer A, Erridge SC, Hegi M, Weller M, Stupp R (2011) Current concepts and management of glioblastoma. Ann Neurol 70:9–21
4. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996
5. Deighton RF, McGregor R, Kemp J, McCulloch J, Whittle IR (2010) Glioma pathophysiology: insights emerging from proteomics. Brain Path 20:691–703
6. Lubec G, Kräpferbauer K, Fountoulakis M (2003) Proteomics in brain research: potentials and limitations. Prog Neurobiol 69: 193–211

7. Persson O, Brynnel U, Levander F, Widegren B, Salford LG, Krogh M (2009) Proteomic expression analysis and comparison of protein and mRNA expression profiles in human malignant gliomas. Proteome Clin Appl 3:83–94

8. MRC Brain Tumour Working Party (1990) Prognostic factors for high grade gliomas: development of a prognostic index. J Neurooncol 9:47

9. Kleinenschmidt-DeMasters Meltesen L, McGavran L, Lillehei KO (2006) Characterisation of glioblastomas in young adults. Brain Pathol 16:273–286

10. Latif AZ, Signorini D, Gregor A, Grant R, Ironside JW, Whittle CC (2011) Effects of different patterns of care? Cancer 103:1234–1244

11. Gorlia T, van den Bent MJ, Hegi ME, Mirimanoff RO, Weller M, Cairncross T, Belanger K, Brandes AA, Allgeier A et al (2008) Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981. Lancet Oncol 9:29–38

12. Lutterbach J, Bartelt S, Mann F, Becker G, Frommhold H, Oertel C (2005) Is older age associated with a worse prognosis due to different patterns of care? Cancer 103:1234–1244

13. Ohgaki H, Kleihues P (2007) Genetic pathways to primary and secondary glioblastoma. Am J Path 170:1445–1453

14. Burton EC, Lamborn KR, Feuerstein BG, Prados M, Scott J, Forsyth P, Passe S, Jenkins RB, Alape AD (2002) Genetic aberrations defined by comparative genomic hybridization distinguishing long-term typical survivors of glioblastoma. Cancer Res 62:6205–6210

15. Schmidt MC, Antweiler S, Urban N, Mueller W, Kuklik A, Meyer-Puttlitz B, Wiestler OD, Lois DN, Fimmers R, von Deede IM, Forsyth P, Passe S, Jenkins RB, Aldape KD (2002) Genetic characterization of long-term survivors of glioblastoma patients correlate with shorter survival. Mol Cancer 10:17

16. Reifenberger G, Brunner U, Levander F, Widegren B, Salford LG, Krogh M (2009) Proteomic expression analysis and comparison of protein and mRNA expression profiles in human malignant gliomas. Proteome Clin Appl 3:83–94

17. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Bae JS, Reardon DA, Joung J, Purcell A, Krieg R, Doolittle EF, Wen PY (2011) IDH1 and IDH2 mutations in gliomas. N Engl J Med 364:1840–1851

18. Ostrac C, Seidenberg EF, Gendron FP, Levy E, Carrier J, Perretault N, Fournier FM, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

19. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

20. Reifenberger G, Seidenberg EF, Gendron FP, Levy E, Carrier J, Perretault N, Boudreau JF (2010) Hepatocyte nuclear factor-4alpha promotes gut neoplasia in mice and protects against the production of reactive oxygen species. Cancer Res 70:9423–9433

21. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

22. Seiffert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

23. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

24. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

25. Seiffert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

26. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

27. Seiffert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

28. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

29. Seiffert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

30. Seiffert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

31. Seiffert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

32. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

33. Seiffert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

34. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

35. Seiffert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M et al (2010) Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142:613–624

36. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

37. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

38. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

39. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

40. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768

41. Santandreu FM, Brell M, Gene AH, Guevara R, Oliver J, Couce ME, Roca P (2008) Differences in mitochondrial function and anti-oxidant systems between regions of human glioma. Cell Physiol Biochem 22:757–768