

# Expression of PGC1 $\alpha$ in glioblastoma multiforme patients

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**Abstract.** Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC1 $\alpha$ ) is a key modulator of mitochondrial biogenesis. It is a coactivator of multiple transcription factors and regulates metabolic processes. However, little is known about the expression and function of PGC1 $\alpha$  in glioblastoma multiforme (GBM), the most prevalent and invasive type of brain tumor. The purpose of the present study was to investigate the biological function, localization and expression of PGC1 $\alpha$  in GBM. It was observed that PGC1 $\alpha$  expression is increased in the tumor cells, and a higher level of expression was observed in the mitochondria. Bioinformatics analyses identified that metabolic and mitochondrial genes were highly expressed in GBM cells, with a high *PGC1 $\alpha$*  mRNA expression. Notably, mitochondrial function-associated genes were highly expressed in cells alongside high *PGC1 $\alpha$*  expression. Collectively, the results of the present study indicate that PGC1 $\alpha$  is associated with mitochondrial dysfunction in GBM and may have a role in tumor pathogenesis and progression.

## Introduction

Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC1 $\alpha$ ) regulates metabolism (1,2), mitochondrial biogenesis and energy homeostasis (3,4). A number of studies have reported PGC1 $\alpha$  as a central regulator of thermogenesis, mitochondrial biogenesis and adaptation to fasting in brown adipose tissue, skeletal muscle, cardiac muscle and the liver (1,5). By contrast, PGC1 $\alpha$  in the central nervous system is less associated with energy state or thermogenesis (6). PGC1 $\alpha$  expression in the central nervous system is high in the embryonic and early postnatal stages, but is decreased during maturation. PGC1 $\alpha$  is expressed mostly by  $\gamma$ -aminobutyric acid-ergic neurons; however, a low level of PGC1 $\alpha$  is also expressed in glia in the mature brain (7). There is a significant association between PGC1 $\alpha$  and the metabolism of reactive oxygen species. PGC1 $\alpha$ -null mice are considerably more sensitive to the neurodegenerative effects of the oxidative stressors 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and kainic acid, which suggests that PGC1 $\alpha$  has a role in cellular antioxidant defense (8).

Numerous clinical studies have reported a significant association between PGC1 $\alpha$  and a number of types of cancer. In breast, colon and ovarian cancer (9-12), a significant decrease in PGC1 $\alpha$  expression accelerated the 'Warburg effect', which allows cancer cells to switch from mitochondrial to glycolytic metabolism to meet the metabolic requirements of proliferation (13). By contrast, increased PGC1 $\alpha$  expression is present in melanoma, with a corresponding decrease in patient survival (14). The role of PGC1 $\alpha$  in a number of cancer types remains unclear and warrants further studies.

Glioblastoma multiforme (GBM) is the most prevalent and invasive type of brain tumor. It aggressively infiltrates and spreads to the surrounding brain tissue via extensive microvascular proliferation. Numerous necrotic areas surrounded by palisading tumor cells are often observed (15). Although novel therapeutic strategies and improved clinical diagnostics have been introduced, GBM remains one of the most fatal diseases (16). An extensive amount of research has been

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performed to determine the mechanisms of unlimited proliferation in GBM, as well as its robust resistance to existing drugs and therapies (17,18). In the present study, the expression of PGC1 $\alpha$  in normal cortical tissues and GBM tissues was compared. The results of the present study indicate that PGC1 $\alpha$  may be a novel biomarker for GBM, as well as a novel target for future GBM therapy development.

## Materials and methods

**Patient samples.** All experiments were performed in accordance with approved guidelines of Chungnam National University Hospital (CNUH; Daejeon, Republic of Korea). The Institutional Review Board of the CNUH approved the experimental protocols and all patients provided written informed consent prior to surgery. A total of 49 patients undergoing tumor resection surgeries at the Department of Neurosurgery, CNUH were enrolled, and pathological diagnoses were confirmed by the Department of Pathology, CNUH via immunohistochemistry. First-time GBM diagnosis was used as the selection criterion, resulting in 26 patient samples that were included in the present study (Table I). The mean age of the patients was 58 years (range, 35 to 74 years). Normal brain tissue samples were obtained from cadavers or from autopsies of surrounding normal brain tissues of consenting GBM patients that underwent surgery (approval no. CNUH 2013-11-006).

**Tissue microarray and immunostaining.** Tissue microarrays (TMA) were used to perform the comparative histological analysis of normal brain and GBM tissues. The paraffin-embedded sample tissues were de-paraffinized and rehydrated in a graded alcohol series. Tissues were retrieved using 0.01 M citrate buffer (pH 6.0) and heated in a microwave vacuum histoprocessor (RHS-1; Milestone Medical, Bergamo, Italy) at a controlled temperature of 121°C for 15 min. Following washing with phosphate-buffered saline (pH 7.4), tissue sections were incubated with anti-PGC1 $\alpha$  antibody (1:200; Santa Cruz Biotechnology, Inc., Dallas, TX, USA; #SC13067) overnight in a humidity chamber at 4°C. Immunohistochemical staining of the tissue sections was performed using avidin-biotin peroxidase complex as previously described (19,20). Additional TMA samples of normal cortex and GBM tissues were obtained from US Biomax, Inc. (Rockville, MD, USA).

All immunostaining was performed with antibodies that detected the N-terminal epitope of PGC1 $\alpha$  (1:200; Santa Cruz Biotechnology, Inc.; #sc-13067). For immunofluorescence analysis, PGC1 $\alpha$  and COX4 (1:200; Cell Signaling Technology, Inc., Danvers, MA, USA; #4D11-B3-E8) were used as above but with either a Cy3-conjugated antibody (1:500; anti-rabbit; GE Healthcare Life Sciences Chalfont, UK; #PA43004) or a Cy2-conjugated secondary antibody (1:200; anti-mouse; GE Healthcare Life Sciences; #PA42002). Cell nuclei were visualized with DAPI, and double-stained sections were visualized using an Axiophot microscope (Carl Zeiss AG, Oberkochen, Germany).

**Bioinformatics.** The mRNA expression of 18,988 probes from 38 GBM cell lines was analyzed using the publicly available

Broad-Novartis Cancer Cell Line Encyclopedia (CCLE) database (<https://portals.broadinstitute.org/ccle/home>) (21). The level of PGC1 $\alpha$  mRNA expression among the 38 GBM cell lines was determined using CCLE. The mRNA expression data was normalized using the RankNormalize module in GenePattern (<http://www.broadinstitute.org/cancer/software/genepattern>). Gene Neighbors and Class Neighbors modules in GenePattern (<http://www.broadinstitute.org/cancer/software/genepattern>) were used to select genes that were closely associated with PGC1 $\alpha$  (22). Hierarchical clustering was performed using complete linkage and Pearson rank-correlation distance with software provided by GenePattern (HierarchicalClustering; version 6). The colors in the heat-maps show the relative gene expression compared to the mean expression, with red being higher and blue lower. From the 18,988 gene set, 100 genes that were most correlated with PGC1 $\alpha$  were selected for classification by Gene Ontology Enrichment Analysis (GO terms) using Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov>) (23). Differentially expressed genes (DEGs) were classified according to GO terms based on their biological process, molecular function or cellular component. DAVID provided an overview of extensive pathways ([www.biocarta.com](http://www.biocarta.com)) in which various genes interacted, as well as the number of DEGs per pathway with a P-value representing gene enrichment. Gene enrichment score with P<0.05 represents a strong association rather than random chance (23). For genes with unknown biological processes, GeneMANIA database (<http://www.genemania.org>) was used to predict their function (24).

**Statistical analysis.** ImageJ software (version 1.47; National Institutes of Health, Bethesda, MD, USA) was used to quantify the optical density (pixels/mm<sup>2</sup>) or the intensity of images. The results from immunohistochemical staining were analyzed by a paired *t*-test between two groups. Data were presented as the mean  $\pm$  standard error. Statistical analyses were performed using the Prism 5.0 software (GraphPad Prism Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference. Data transformation (log conversion) selection and statistical analyses were performed with either the Microsoft Excel 11.0 (Microsoft Corporation, Redmond, WA, USA) or Prism 5.0 software.

## Results

**PGC1 $\alpha$  is highly and variably expressed in GBM patients.** To determine the association between PGC1 $\alpha$  and GBM, levels of PGC1 $\alpha$  protein in GBM and control (normal cortex) tissues were compared using publicly available TMAs from US Biomax, Inc. (Fig. 1). PGC1 $\alpha$  was weakly detectable in the nuclei of cortical tissues in the control, whereas it was highly and sporadically expressed throughout the GBM tissues. Furthermore, PGC1 $\alpha$  was mostly expressed within the cytoplasm with pale nucleic density (Fig. 1A). Bright-field immunohistochemical analysis of TMA images using a densitometer revealed that PGC1 $\alpha$  expression varied between tumor samples (Fig. 1B).

For additional validation, PGC1 $\alpha$  mRNA levels were determined in GBM cell lines (n=38) using the Broad-Novartis CCLE database (21). Comparative analysis of PGC1 $\alpha$

Table I. Patient demographics and tumor characteristics.

| Case no. | Age <sup>a</sup> (years) | Gender | Pathological diagnosis | Ki-67 (%) | Resection area        |
|----------|--------------------------|--------|------------------------|-----------|-----------------------|
| 1        | 64                       | M      | GBM                    | 20        | Left, parietal lobe   |
| 2        | 56                       | F      | GBM                    | 20        | Right, frontal lobe   |
| 3        | 58                       | M      | GBM                    | 20        | Left, temporal lobe   |
| 4        | 60                       | M      | GBM                    | 20        | Left, temporal lobe   |
| 5        | 40                       | M      | GBM                    | 20        | Left, frontal lobe    |
| 6        | 35                       | M      | GBM                    | 20        | Left, frontal lobe    |
| 7        | 56                       | F      | GBM                    | 20        | Right, frontal lobe   |
| 8        | 63                       | M      | GBM                    | 20        | Right, parietal lobe  |
| 9        | 72                       | M      | GBM                    | 20        | Right, occipital lobe |
| 10       | 66                       | F      | GBM                    | 40        | Left, parietal lobe   |
| 11       | 49                       | F      | GBM                    | 15        | Left, temporal lobe   |
| 12       | 44                       | M      | Giant cell GBM         | 40        | Right, frontal lobe   |
| 13       | 77                       | F      | GBM                    | 40        | Right frontal lobe    |
| 14       | 55                       | M      | GBM cerebri            | 20        | Right, frontal lobe   |
| 15       | 71                       | F      | GBM                    | 90        | Right, parietal lobe  |
| 16       | 51                       | M      | GBM                    | 30        | Left, temporal lobe   |
| 17       | 56                       | M      | GBM                    | 20        | Right, midbrain       |
| 18       | 61                       | M      | GBM                    | 30        | Left, temporal lobe   |
| 19       | 52                       | F      | GBM                    | 30        | Left, parietal lobe   |
| 20       | 45                       | M      | GBM                    | 40        | Right, temporal lobe  |
| 21       | 71                       | F      | GBM                    | 30        | Right, frontal lobe   |
| 22       | 55                       | M      | GBM                    | 20        | Left, temporal lobe   |
| 23       | 52                       | M      | GBM                    | 50        | Left, parietal lobe   |
| 24       | 57                       | M      | GBM                    | 40        | Right, temporal lobe  |
| 25       | 74                       | M      | GBM                    | 40        | Right, parietal lobe  |
| 26       | 74                       | M      | GBM                    | 25        | Left, insular         |

<sup>a</sup>Mean, 58.23 years. GBM, glioblastoma multiforme.

expression in GBM and five other types of cancer, including liver, ovarian, endometrial, breast and prostate carcinoma revealed that although there were variations in *PGC1α* mRNA expression between the GBM cell lines (Fig. 1D), the level of expression was increased in GBM compared to other cancer cell lines (Fig. 1C). Overall, these data demonstrate that *PGC1α* expression was increased in a subpopulation of GBM cells.

*PGC1α is localized to the mitochondria in GBM.* As a transcriptional coactivator, *PGC1α* is reported to be localized in the nuclei of the normal cortex (25). However, immunofluorescence analysis demonstrated localization of *PGC1α* in the perinuclear or cytoplasmic areas of GBM tissues (Fig. 2A). To confirm the subcellular localization of *PGC1α*, double staining with anti-*PGC1α* and anti-COX4 (a mitochondrial marker) antibodies was employed. There was a certain level of colocalization of *PGC1α* and COX4, thereby indicating that *PGC1α* was expressed in the mitochondria in GBM in addition to the perinuclear or cytoplasmic areas (Fig. 2B).

*Gene Neighbors of PGC1α.* Bioinformatics analysis of *PGC1α*-associated genes was performed. *PGC1α* mRNA

expression levels detected in the GBM cell lines (n=38; Table II) ranged from 3.71 (log<sub>2</sub>) to 8.83 (log<sub>2</sub>), which corresponds to a fold-change of 2.38. A total of 100 genes that were strongly correlated with *PGC1α* were selected using Gene Neighbors (Fig. 3A) and classified using DAVID (23). Genes with significant differences (P<0.05) were classified into two groups based on GO terms: Biological process and cellular components (Tables III and IV). Genes highly expressed in GBM cell lines were largely associated with the generation of metabolite precursors and energy (e.g., the hexose or monosaccharide metabolic processes), oxidation reduction (e.g., mitochondrial electron transport, nicotinamide adenine dinucleotide to ubiquinone and the oxidoreduction coenzyme metabolic process), energy derivation by the oxidation of organic compounds [e.g., acetyl-CoA metabolic and catabolic processes, oxidative phosphorylation, tricarboxylic acid (TCA) cycle, aerobic respiration and glycolysis, and coenzyme metabolic and catabolic processes (e.g., cofactor catabolic process) (Fig. 3B). Notably, highly expressed genes were associated with the mitochondria (e.g., mitochondrial membrane, mitochondrial matrix and mitochondrial respiratory chain), organelle membranes (e.g., organelle inner membrane) and the cellular envelope (Fig. 3C). This observation is in agreement

Table II. List of GBM cell lines.

| GBM cell lines | PGC1 $\alpha$ mRNA |
|----------------|--------------------|
| LNZ308         | 8.83               |
| LN464          | 8.79               |
| DBTRG05MG      | 8.65               |
| LN235          | 8.40               |
| SNU626         | 7.65               |
| GB1            | 7.45               |
| YKG1           | 6.64               |
| U343           | 6.59               |
| LN428          | 6.52               |
| SNB19          | 6.49               |
| GMS10          | 6.27               |
| LN340          | 6.17               |
| KNS81          | 6.11               |
| 8MGBA          | 5.72               |
| SNU201         | 5.63               |
| T98G           | 5.53               |
| YH13           | 5.33               |
| LN382          | 5.19               |
| CAS1           | 5.11               |
| U178           | 4.71               |
| SF295          | 4.69               |
| SNU1105        | 4.62               |
| SNU489         | 4.60               |
| DKMG           | 4.42               |
| BECKER         | 4.30               |
| 42MGBA         | 4.29               |
| KG1C           | 4.22               |
| A172           | 4.17               |
| LN443          | 4.13               |
| LN215          | 4.09               |
| AM38           | 4.04               |
| LN18           | 4.04               |
| M059K          | 4.02               |
| LN229          | 4.00               |
| KNS60          | 4.00               |
| SF172          | 3.84               |
| SNU466         | 3.74               |
| KS1            | 3.71               |

GBM, glioblastoma multiforme; PGC1 $\alpha$ , proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$ .

with the finding that PGC1 $\alpha$  is localized in the mitochondria in GBM as previously described.

*PGC1 $\alpha$  expression is highly correlated with mitochondrial function in GBM.* Two-way hierarchical clustering of targeted gene sets was performed between five GBM cell lines with the highest (LNZ308, LN464, DBTRG05MG, LN235 and SNU626) and lowest levels (LN229, KNS60, SF172, SNU466 and KS1) of *PGC1 $\alpha$*  expression. The expression of TCA cycle- ( $P < 0.0001$ ),

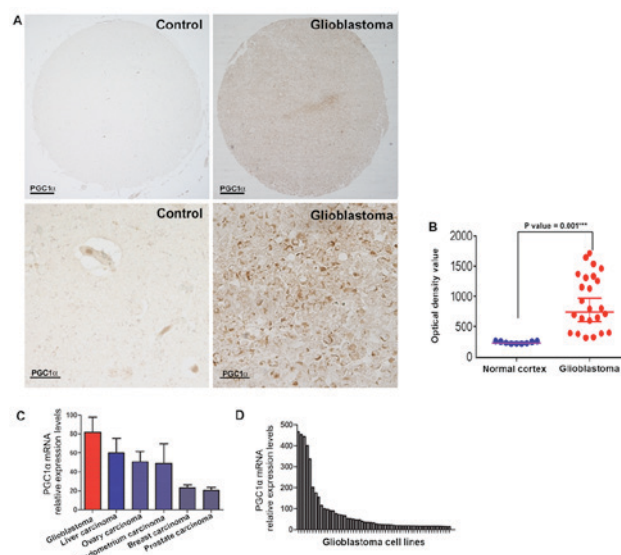


Figure 1. Expression level of PGC1 $\alpha$  in glioblastoma multiforme and normal cortex. (A) Representative immunohistochemical analysis of PGC1 $\alpha$  expression from the US Biomax, Inc. TMA database. Scale bar: 100  $\mu$ m (upper panels) and 20  $\mu$ m (lower panels). (B) Corrected optical density values of PGC1 $\alpha$  in normal cortex tissues and glioblastoma tissues from the US Biomax, Inc. TMA database (unpaired t-test,  $\pm$  SEM; \*\*\* $P < 0.001$ ). (C) Relative mRNA expression of *PGC1 $\alpha$*  across various cancer types in the CCLE database. Error bars: mean  $\pm$  SEM in glioblastoma (n=38), liver carcinoma (n=28), ovary carcinoma (n=52), endometrium carcinoma (n=27), breast carcinoma (n=59) and prostate carcinoma (n=8). (D) Relative expression values of *PGC1 $\alpha$*  mRNA among 38 glioblastoma multiforme cell lines in the CCLE database. CCLE, cancer cell line encyclopedia; PGC1 $\alpha$ , peroxisome proliferator-activated receptor  $\gamma$ , coactivator 1 $\alpha$ ; TMA, tissue microarrays; SEM, standard error of the mean.

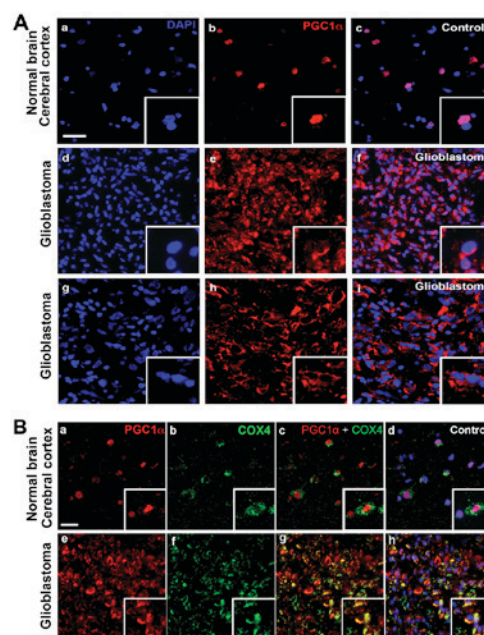


Figure 2. Colocalization of PGC1 $\alpha$  with COX4 in GBM. (A) PGC1 $\alpha$  localization in glioblastoma multiforme and normal cortex was analyzed using tissue microarrays. PGC1 $\alpha$  expression in the normal cortex was localized to the nucleus, but perinuclear and cytoplasmic expression of PGC1 $\alpha$  was observed in GBM. (B) Representative co-immunofluorescence staining of PGC1 $\alpha$  (red) and COX4 (green) with counter-staining with DAPI (blue) in the normal cortex and GBM. PGC1 $\alpha$ -positive cells were primarily co-labeled with the mitochondrial marker COX4 in GBM. COX4, cytochrome c oxidase subunit 4; GBM, glioblastoma multiforme; PGC1 $\alpha$ , peroxisome proliferator-activated receptor  $\gamma$ .



Table III. List of Gene Neighbors of peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  differentially expressed in glioblastoma multiforme cells.

| Gene symbol   | Description  |
|---|--|
| Generation of precursor metabolites and energy      |  |
| <i>ATP5J</i>  | ATP synthase, H <sup>+</sup> transporting, mitochondrial Fo complex, subunit F6          |
| <i>ATP5B</i>  | ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, $\beta$ polypeptide |
| <i>NDUFA1</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 1, 7.5 kDa                        |
| <i>NDUFA4</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 4, 9 kDa                          |
| <i>NDUFA7</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 7, 14.5 kDa                       |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>GYG2</i>   | Glycogenin 2   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| <i>MCHR1</i>  | Melanin-concentrating hormone receptor 1   |
| <i>OGDHL</i>  | Oxoglutarate dehydrogenase-like  |
| <i>PDHA1</i>  | Pyruvate dehydrogenase (lipoamide) $\alpha$ 1  |
| Oxidation reduction                                 |  |
| <i>NDUFA1</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 1, 7.5 kDa                        |
| <i>NDUFA4</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 4, 9 kDa                          |
| <i>NDUFA7</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 7, 14.5 kDa                       |
| <i>AIFM1</i>  | Apoptosis-inducing factor, mitochondrion-associated, 1                                   |
| <i>CYP27A1</i>                                      | Cytochrome p450, family 27, subfamily A, polypeptide 1                                   |
| <i>COX5A</i>  | Cytochrome c oxidase subunit Va  |
| <i>HCCS</i>   | Holocytochrome c synthase  |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| <i>OGDHL</i>  | Oxoglutarate dehydrogenase-like  |
| <i>PIPOX</i>  | Pipecolic acid oxidase   |
| <i>PRODH</i>  | Proline dehydrogenase (oxidase) 1  |
| <i>PDHA1</i>  | Pyruvate dehydrogenase (lipoamide) $\alpha$ 1  |
| Energy derivation by oxidation of organic compounds |  |
| <i>NDUFA1</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 1, 7.5 kDa                        |
| <i>NDUFA4</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 4, 9 kDa                          |
| <i>NDUFA7</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 7, 14.5 kDa                       |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>GYG2</i>   | Glycogenin 2   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Cellular respiration                                |  |
| <i>NDUFA1</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 1, 7.5 kDa                        |
| <i>NDUFA4</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 4, 9 kDa                          |
| <i>NDUFA7</i>                                       | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 7, 14.5 kDa                       |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Acetyl-CoA metabolic process                        |  |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>ACSS1</i>  | Acyl-CoA synthetase short-chain family member 1  |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Coenzyme metabolic process                          |  |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>ACSS1</i>  | Acyl-CoA synthetase short-chain family member 1  |

Table III. Continued.

| Gene symbol   | Description  |
|---|--|
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Oxidation phosphorylation                               |  |
| <i>ATP5J</i>  | ATP synthase, H <sup>+</sup> transporting, mitochondrial Fo complex, subunit F6          |
| <i>ATP5B</i>  | ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, $\beta$ polypeptide |
| <i>NDUFA1</i>   | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 1, 7.5 kDa                        |
| <i>NDUFA4</i>   | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 4, 9 kDa                          |
| <i>NDUFA7</i>   | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 7, 14.5 kDa                       |
| Cofactor metabolic process                              |  |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>ACSS1</i>  | Acyl-CoA synthetase short-chain family member 1  |
| <i>COQ9</i>   | Coenzyme Q9 homolog ( <i>S. cerevisiae</i> )   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| <i>PIPOX</i>  | Pipecolic acid oxidase   |
| Acetyl-CoA catabolic process                            |  |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Tricarboxylic acid cycle                                |  |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Coenzyme catabolic process                              |  |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Cofactor catabolic process                              |  |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Aerobic respiration                                     |  |
| <i>ACO2</i>   | Aconitase 2, mitochondrial   |
| <i>IDH3A</i>  | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                                  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| Hexose metabolic process                                |  |
| <i>PFKFB3</i>   | 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3                                    |
| <i>GYG2</i>   | Glycogenin 2   |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| <i>OGDHL</i>  | Oxoglutarate dehydrogenase-like  |
| <i>PDHA1</i>  | Pyruvate dehydrogenase (lipoamide) $\alpha$ 1  |
| Mitochondrial electron transport,<br>NADH to ubiquinone |  |
| <i>NDUFA1</i>   | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 1, 7.5 kDa                        |
| <i>NDUFA4</i>   | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 4, 9 kDa                          |
| <i>NDUFA7</i>   | NADH dehydrogenase (ubiquinone) 1 $\alpha$ subcomplex, 7, 14.5 kDa                       |
| Glycolysis  |  |
| <i>MDH1</i>   | Malate dehydrogenase 1, NAD (soluble)  |
| <i>OGDHL</i>  | Oxoglutarate dehydrogenase-like  |
| <i>PDHA1</i>  | Pyruvate dehydrogenase (lipoamide) $\alpha$ 1  |

Table III. Continued.

| Gene symbol                               | Description   |
|---|---|
| Monosaccharide metabolic process          |   |
| <i>PFKFB3</i>                             | 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3                             |
| <i>GYG2</i>                               | Glycogenin 2  |
| <i>MDH1</i>                               | Malate dehydrogenase 1, NAD (soluble)   |
| <i>OGDHL</i>                              | Oxoglutarate dehydrogenase-like   |
| <i>PDHA1</i>                              | Pyruvate dehydrogenase (lipoamide) $\alpha$ 1                                     |
| Oxidoreduction coenzyme metabolic process |   |
| <i>COQ9</i>                               | Coenzyme Q9 homolog ( <i>S. cerevisiae</i> )                                      |
| <i>IDH3A</i>                              | Isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) $\alpha$                           |
| <i>MDH1</i>                               | Malate dehydrogenase 1, NAD (soluble)   |
| Unknown biological process                |   |
| <i>CEND1</i>                              | Cell cycle exit and neuronal differentiation 1                                    |
| <i>COX7B</i>                              | Cytochrome c oxidase subunit VIIb   |
| <i>TMCC2</i>                              | Transmembrane and coiled-coil domain family 2                                     |
| <i>SOX13</i>                              | SRY (sex determining region Y)-box 13   |
| <i>BTBD3</i>                              | BTB (POZ) domain containing 3   |
| <i>ZNF222</i>                             | Zinc finger protein 222   |
| <i>DCUN1D2</i>                            | DCN1, defective in cullin neddylation 1, domain containing 2                      |
| <i>MFS2A</i>                              | Major facilitator superfamily domain containing 2A                                |
| <i>CX3CL1</i>                             | Chemokine (C-X3-C motif) ligand 1   |
| <i>GSTM4</i>                              | Glutathione S-transferase mu 4  |
| <i>PIGA</i>                               | Phosphatidylinositol glycan anchor biosynthesis, class A                          |
| <i>ITPKB</i>                              | Inositol-trisphosphate 3-kinase B   |
| <i>TSPAN16</i>                            | Tetraspanin 16  |
| <i>CHCHD3</i>                             | Coiled-coil-helix-coiled-coil-helix domain containing 3                           |
| <i>APOO</i>                               | Apolipoprotein O  |
| <i>AKAP11</i>                             | A kinase (PRKA) anchor protein 11   |
| <i>NEBL</i>                               | Nebulette   |
| <i>SCUBE3</i>                             | Signal peptide, CUB domain, EGF-like 3  |
| <i>RRAGD</i>                              | Ras-related GTP binding D   |
| <i>IGHV1-2</i>                            | Immunoglobulin heavy variable 1-2   |
| <i>RRAGD</i>                              | Ras-related GTP binding D   |
| <i>TRIM2</i>                              | Tripartite motif containing 2   |
| <i>TLE6</i>                               | Transducin-like enhancer of split 6 (E(sp1) homolog, <i>Drosophila</i> )          |
| <i>LINC00461</i>                          | Long intergenic non-protein coding RNA 461  |
| <i>SLC25A25</i>                           | Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25    |
| <i>SLC25A11</i>                           | Solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11 |
| <i>IVNS1ABP</i>                           | Influenza virus NS1A binding protein  |
| <i>HEY1</i>                               | Hairy/enhancer-of-split related with YRPW motif 1                                 |
| <i>NDRG2</i>                              | NDRG family member 2  |
| <i>COX5B</i>                              | Cytochrome c oxidase subunit Vb   |
| <i>MRPL34</i>                             | Mitochondrial ribosomal protein L34   |
| <i>STK32A</i>                             | Serine/threonine kinase 32A   |
| <i>MEGF8</i>                              | Multiple EGF-like-domains 8   |
| <i>ATP1A1</i>                             | ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, $\alpha$ 1 polypeptide      |
| <i>RBPMS2</i>                             | RNA binding protein with multiple splicing 2                                      |
| <i>LPL</i>                                | Lipoprotein lipase  |
| <i>FURIN</i>                              | Furin (paired basic amino acid cleaving enzyme)                                   |
| <i>ASAHL</i>                              | N-acylsphingosine amidohydrolase (acid ceramidase) 1                              |
| <i>KLHL15</i>                             | Kelch-like family member 15   |
| <i>BTBD1</i>                              | BTB (POZ) domain containing 1   |
| <i>PTCD3</i>                              | Pentatricopeptide repeat domain 3   |

Table III. Continued.

| Gene symbol         | Description   |
|---------------------|---|
| <i>RBM38</i>        | RNA binding motif protein 38  |
| <i>LYNX1</i>        | Ly6/neurotoxin 1  |
| <i>EFHA1</i>        | Mitochondrial calcium uptake 2  |
| <i>NCOA1</i>        | Nuclear receptor coactivator 1  |
| <i>KIF13B</i>       | Kinesin family member 13B   |
| <i>FAM199X</i>      | Family with sequence similarity 199, X-linked                             |
| <i>RPRM</i>         | Reprimo, TP53 dependent G2 arrest mediator candidate                      |
| <i>ZNF462</i>       | Zinc finger protein 462   |
| <i>ANXA13</i>       | Annexin A13   |
| <i>SPG20OS</i>      | SPG20 opposite strand   |
| <i>GPR98</i>        | G protein-coupled receptor 98   |
| <i>GK</i>           | Glycerol kinase   |
| <i>UCK1</i>         | Uridine-cytidine kinase 1   |
| <i>LNX2</i>         | Ligand of numb-protein X 2  |
| <i>SPG20</i>        | Spastic paraplegia 20 (Troyer syndrome)                                   |
| <i>WNK3</i>         | WNK lysine deficient protein kinase 3                                     |
| <i>LOC100506108</i> | LOC100506108  |
| <i>GCNT2</i>        | Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group) |
| <i>SLC31A1</i>      | Solute carrier family 31 (copper transporter), member 1                   |
| <i>OSTM1</i>        | Osteopetrosis associated transmembrane protein 1                          |
| <i>TMF1</i>         | TATA element modulatory factor 1  |
| <i>TSPAN3</i>       | Tetraspanin 3   |
| <i>COL4A3</i>       | Collagen, type IV, $\alpha$ 3 (Goodpasture antigen)                       |
| <i>GPM6B</i>        | Glycoprotein M6B  |
| <i>PELI2</i>        | Pellino E3 ubiquitin protein ligase family member 2                       |
| <i>LOC401431</i>    | LOC401431   |
| <i>UBAC1</i>        | UBA domain containing 1   |
| <i>ATG4D</i>        | Autophagy related 4D, cysteine peptidase                                  |
| <i>COMMD6</i>       | COMM domain containing 6  |
| <i>FAM65B</i>       | Family with sequence similarity 65, member B                              |
| <i>TMEM2</i>        | Transmembrane protein 2   |
| <i>ASB9</i>         | Ankyrin repeat and SOCS box containing 9                                  |
| <i>BCAM</i>         | Basal cell adhesion molecule (Lutheran blood group)                       |
| <i>KIF16B</i>       | Kinesin family member 16B   |
| <i>CHKA</i>         | Choline kinase $\alpha$   |
| <i>PPM1E</i>        | Protein phosphatase, Mg <sup>2+</sup> /Mn <sup>2+</sup> dependent, 1E     |
| <i>CA2</i>          | Carbonic anhydrase II   |

oxidative phosphorylation (OXPHOS)-(P<0.0001) and lipogenesis-associated genes (P<0.01) was significantly increased in the *PGC1 $\alpha$* -upregulated cells compared with the *PGC1 $\alpha$* -downregulated cells (Fig. 4A-C). Furthermore, the expression of antioxidant-associated genes was significantly increased in the *PGC1 $\alpha$* -upregulated cell lines compared with the *PGC1 $\alpha$* -downregulated cell lines (Fig. 4D; P<0.0001). Taken together, the data in Figs. 3 and 4 suggest that metabolic and mitochondrial genes were highly expressed in parallel with *PGC1 $\alpha$* . Notably, genes associated with mitochondrial functions, including TCA cycle, OXPHOS, lipogenesis and antioxidant genes, were highly expressed in cells with high *PGC1 $\alpha$*  levels (Fig. 4), which corroborates the results from

a recent study (26) and the colocalization data as previously described in the present study.

*Class Neighbors of PGC1 $\alpha$  up- and downregulated GBM cell lines.* Bioinformatics analysis using Class Neighbors yielded two classes of GBM cell lines. Class A contained the ten most *PGC1 $\alpha$* -upregulated GBM cell lines, and class B contained the ten most *PGC1 $\alpha$* -downregulated GBM cell lines (Fig. 5A). Out of a total of 18,988 probe sets, 100 genes that were most strongly correlated with classes A and B and most highly expressed were selected. DAVID analysis classified these genes into three groups based on GO terms: i) Biological process, ii) molecular function and iii) cellular components (Fig. 5B and C;



Table IV. Annotated summary of Gene Neighbors of peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$ .

| Functional role  | Genes | P-value               | -Log (P-value) |
|--|-------|-----------------------|----------------|
| Biological process                                     |       |                       |                |
| Generation of precursor metabolites and energy         | 12    | 5.50x10 <sup>7</sup>  | 6.26           |
| Oxidation reduction                                    | 13    | 9.60x10 <sup>5</sup>  | 4.02           |
| Energy derivation by oxidation of organic compounds    | 7     | 9.80x10 <sup>5</sup>  | 4.01           |
| Cellular respiration                                   | 6     | 1.40x10 <sup>4</sup>  | 3.85           |
| Acetyl-CoA metabolic process                           | 4     | 5.40x10 <sup>4</sup>  | 3.27           |
| Coenzyme metabolic process                             | 6     | 1.20x10 <sup>3</sup>  | 2.92           |
| Oxidative phosphorylation                              | 5     | 1.60x10 <sup>3</sup>  | 2.80           |
| Cofactor metabolic process                             | 6     | 3.40x10 <sup>3</sup>  | 2.47           |
| Tricarboxylic acid cycle                               | 3     | 6.20x10 <sup>3</sup>  | 2.21           |
| Acetyl-CoA catabolic process                           | 3     | 6.20x10 <sup>3</sup>  | 2.21           |
| Coenzyme catabolic process                             | 3     | 7.90x10 <sup>3</sup>  | 2.10           |
| Cofactor catabolic process                             | 3     | 1.10x10 <sup>2</sup>  | 1.96           |
| Aerobic respiration                                    | 3     | 1.40x10 <sup>2</sup>  | 1.85           |
| Hexose metabolic process                               | 5     | 1.70x10 <sup>2</sup>  | 1.77           |
| Mitochondrial electron transport, NADH to ubiquinone   | 3     | 2.00x10 <sup>2</sup>  | 1.70           |
| Glycolysis   | 3     | 2.50x10 <sup>2</sup>  | 1.60           |
| Monosaccharide metabolic process                       | 5     | 2.80x10 <sup>2</sup>  | 1.55           |
| Oxidoreduction coenzyme metabolic process              | 3     | 3.00x10 <sup>2</sup>  | 1.52           |
| Cellular component                                     |       |                       |                |
| Mitochondrial part                                     | 22    | 3.40x10 <sup>12</sup> | 11.47          |
| Mitochondrion  | 25    | 1.20x10 <sup>9</sup>  | 8.92           |
| Mitochondrial envelope                                 | 16    | 5.90x10 <sup>9</sup>  | 8.23           |
| Mitochondrial inner membrane                           | 14    | 9.20x10 <sup>9</sup>  | 8.04           |
| Mitochondrial membrane                                 | 15    | 2.20x10 <sup>8</sup>  | 7.66           |
| Organelle inner membrane                               | 14    | 2.20x10 <sup>8</sup>  | 7.66           |
| Organelle envelope                                     | 16    | 9.80x10 <sup>7</sup>  | 6.01           |
| Envelope   | 16    | 1.00x10 <sup>6</sup>  | 6.00           |
| Mitochondrial lumen                                    | 9     | 3.20x10 <sup>5</sup>  | 4.49           |
| Mitochondrial matrix                                   | 9     | 3.20x10 <sup>5</sup>  | 4.49           |
| Organelle membrane                                     | 18    | 6.40x10 <sup>5</sup>  | 4.19           |
| Mitochondrial membrane part                            | 6     | 6.00x10 <sup>4</sup>  | 3.22           |
| Mitochondrial respiratory chain                        | 4     | 5.20x10 <sup>3</sup>  | 2.28           |
| Respiratory chain                                      | 4     | 8.00x10 <sup>3</sup>  | 2.10           |
| Respiratory chain complex I                            | 3     | 2.20x10 <sup>2</sup>  | 1.66           |
| Mitochondrial respiratory chain complex I              | 3     | 2.20x10 <sup>2</sup>  | 1.66           |
| NADH dehydrogenase complex                             | 3     | 2.20x10 <sup>2</sup>  | 1.66           |
| Cell surface   | 6     | 4.20x10 <sup>2</sup>  | 1.38           |
| Mitochondrial proton-transporting ATP synthase complex | 2     | 9.90x10 <sup>2</sup>  | 1.00           |

The dataset of significantly changed genes were identified using the Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov>) ( $P < 0.05$ ). ATP, adenosine triphosphate; NADH, reduced Nicotinamide adenine dinucleotide.

Tables V-VIII). GeneMANIA database analysis resulted in the identification of 52 genes with previously unknown biological interactions with *PGC1 $\alpha$* , including necdin (*NDN*).

In addition, when genes were analyzed according to cell signaling pathway (BioCarta database), 3 signaling pathways in class A and 5 in class B were identified as statistically significant ( $P < 0.05$ ; Table IX). The results of the present study demonstrate that class A genes play roles in signaling pathways

associated with metabolic and mitochondrial electron transport and that class B genes are involved in signaling pathways associated with differentiation and immune function.

## Discussion

The objective of the present study was to investigate the association between aberrant expression of *PGC1 $\alpha$*  and GBM, and

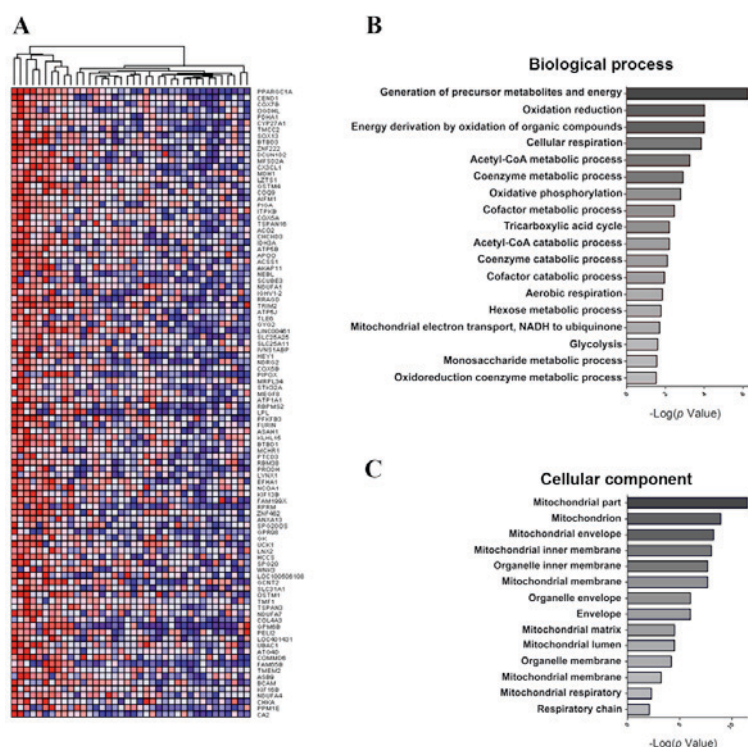


Figure 3. Bioinformatics analysis of *PGC1 $\alpha$* -associated genes in GBM cell lines. (A) Hierarchical clustering of *PGC1 $\alpha$*  Gene Neighbors in GBM cell lines. GBM cells are arranged in decreasing order of *PGC1 $\alpha$*  mRNA expression by Pearson distance. Colors in the heat-map represent expression relative to the mean expression value, with red indicating higher expression and blue indicating lower expression. Gene neighbors of *PGC1 $\alpha$*  are displayed in the right column. Gene Neighbors of *PGC1 $\alpha$*  were characterized as either (B) biological processes or (C) cellular components by gene ontology enrichment analysis. GBM, glioblastoma multiforme; PGC1 $\alpha$ , peroxisome proliferator-activated receptor  $\gamma$ , coactivator 1 $\alpha$ .

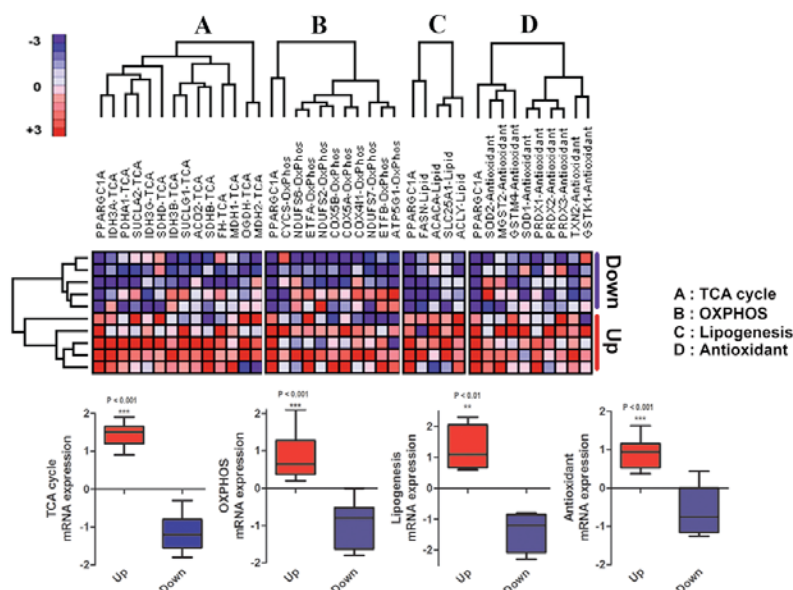


Figure 4. Two-way hierarchical clustering of target gene sets previously reported to be associated with *PGC1 $\alpha$*  in cancer. (A) TCA cycle genes ( $^{***}P < 0.001$ ), (B) OXPHOS genes ( $^{***}P < 0.001$ ), (C) Lipogenesis genes ( $^{**}P < 0.01$ ) and (D) Antioxidant genes ( $^{***}P < 0.001$ ) are differentially expressed among the top five *PGC1 $\alpha$*  up- and downregulated GBM cell lines. The top five GBM cell lines are LN2308, LN464, DBTRG05MG, LN235 and SNU626. The bottom five GBM cell lines are LN229, KNS60, SF172, SNU466 and KS1. Color in the heat-maps displays expression relative to the mean expression value, with red indicating higher expression and blue lower expression. Colors are displayed in a score ladder from red to blue (+3 to -3, upper left panel). The obtained values were analyzed statistically by paired t-test. GBM, glioblastoma multiforme; OXPHOS, oxidative phosphorylation; PGC1 $\alpha$ , peroxisome proliferator-activated receptor  $\gamma$ , coactivator 1 $\alpha$ ; TCA, tricarboxylic acid.

the role PGC1 $\alpha$  may have in patient survival. Protein level data demonstrated that PGC1 $\alpha$  expression was increased in a subpopulation of tumor cells, although there were variations

between different GBM cell lines and patients. PGC1 $\alpha$  localization was identified to differ between GBM tissues and the normal cortex (Fig. 2). These results corroborated

Table V. List of class A genes highly expressed in peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$ -upregulated glioblastoma multiforme cells.

| Gene                    | Description  | Score | P-value             | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|-------------------------|--|-------|---------------------|-------------|----------------------|------------------------|
| Developmental processes |  |       |                     |             |                      |                        |
| <i>CLEC2B</i>           | C-type lectin domain family 2, member B              | 2.63  | 3.2x10 <sup>3</sup> | 1.44        | 5.88                 | 4.09                   |
| <i>EFHD1</i>            | EF-hand domain family, member D1                     | 2.22  | 4.4x10 <sup>2</sup> | 1.31        | 6.03                 | 4.61                   |
| <i>EPHA3</i>            | EPH receptor A3                                      | 2.45  | 1.9x10 <sup>2</sup> | 1.39        | 5.33                 | 3.83                   |
| <i>HHIPL2</i>           | HHIP-like 2  | 3.52  | 2.6x10 <sup>3</sup> | 1.21        | 5.49                 | 4.53                   |
| <i>MAMDC2</i>           | MAM domain containing 2                              | 2.49  | 2.2x10 <sup>2</sup> | 1.42        | 6.76                 | 4.77                   |
| <i>POU3F2</i>           | POU class 3 homeobox 2                               | 2.54  | 2.6x10 <sup>2</sup> | 1.40        | 7.20                 | 5.15                   |
| <i>BHLHE41</i>          | Basic helix-loop-helix family, member e41            | 2.92  | 6.2x10 <sup>3</sup> | 1.26        | 6.14                 | 4.89                   |
| <i>CDH6</i>             | Cadherin 6, type 2, K-cadherin (fetal kidney)        | 2.80  | 1.1x10 <sup>2</sup> | 1.35        | 5.83                 | 4.31                   |
| <i>CELSR2</i>           | Cadherin, EGF LAG seven-pass G-type receptor 2       | 2.80  | 1.2x10 <sup>2</sup> | 1.21        | 7.78                 | 6.42                   |
| <i>CXCR4</i>            | Chemokine (C-X-C motif) receptor 4                   | 2.55  | 1.5x10 <sup>2</sup> | 1.44        | 6.04                 | 4.20                   |
| <i>CNIH3</i>            | Cornichon family AMPA receptor auxiliary protein 3   | 2.41  | 3.3x10 <sup>2</sup> | 1.41        | 7.22                 | 5.11                   |
| <i>CCNA1</i>            | Cyclin A1  | 2.56  | 1.1x10 <sup>2</sup> | 1.32        | 5.71                 | 4.32                   |
| <i>FABP7</i>            | Fatty acid binding protein 7, brain                  | 2.26  | 3.1x10 <sup>2</sup> | 1.57        | 6.87                 | 4.38                   |
| <i>FBLN1</i>            | Fibulin 1  | 2.62  | 1.9x10 <sup>2</sup> | 1.27        | 7.35                 | 5.78                   |
| <i>FOXA2</i>            | Forkhead box A2                                      | 2.18  | 6.2x10 <sup>2</sup> | 1.36        | 5.56                 | 4.09                   |
| <i>GPM6B</i>            | Glycoprotein M6B                                     | 2.14  | 4.8x10 <sup>2</sup> | 1.46        | 7.60                 | 5.19                   |
| <i>HES1</i>             | Hairy and enhancer of split 1, ( <i>Drosophila</i> ) | 3.29  | 4.2x10 <sup>3</sup> | 1.21        | 8.42                 | 6.98                   |
| <i>HEY1</i>             | Hairy/enhancer-of-split related with YRPW motif 1    | 2.49  | 2.3x10 <sup>2</sup> | 1.28        | 8.16                 | 6.39                   |
| <i>IRX1</i>             | Iroquois homeobox 1                                  | 2.81  | 8.4x10 <sup>3</sup> | 1.48        | 6.61                 | 4.47                   |
| <i>JAG1</i>             | Jagged 1   | 3.16  | 6.0x10 <sup>3</sup> | 1.22        | 7.89                 | 6.48                   |
| <i>MYL5</i>             | Myosin, light chain 5, regulatory                    | 3.19  | 5.6x10 <sup>3</sup> | 1.25        | 6.73                 | 5.40                   |
| <i>NRG2</i>             | Neuregulin 2   | 2.73  | 1.4x10 <sup>2</sup> | 1.22        | 4.99                 | 4.09                   |
| <i>NRP2</i>             | Neuropilin 2   | 2.75  | 1.2x10 <sup>2</sup> | 1.25        | 6.74                 | 5.40                   |
| <i>PTH1H</i>            | Parathyroid hormone-like hormone                     | 2.46  | 1.9x10 <sup>2</sup> | 1.42        | 6.77                 | 4.75                   |
| <i>PRICKLE2</i>         | Prickle homolog 2 ( <i>Drosophila</i> )              | 2.46  | 2.3x10 <sup>2</sup> | 1.22        | 8.15                 | 6.70                   |
| <i>SALL1</i>            | Sal-like 1 ( <i>Drosophila</i> )                     | 2.41  | 2.5x10 <sup>2</sup> | 1.36        | 6.83                 | 5.04                   |
| <i>SCUBE3</i>           | Signal peptide, CUB domain, EGF-like 3               | 2.63  | 3.6x10 <sup>3</sup> | 1.34        | 7.76                 | 5.78                   |
| <i>TLR4</i>             | Toll-like receptor 4                                 | 2.82  | 9.8x10 <sup>3</sup> | 1.36        | 6.29                 | 4.61                   |
| Signal transduction     |  |       |                     |             |                      |                        |
| <i>EPHA3</i>            | EPH receptor A3                                      | 2.45  | 1.9x10 <sup>2</sup> | 1.39        | 5.33                 | 3.83                   |
| <i>GPR56</i>            | G protein-coupled receptor 56                        | 3.00  | 9.4x10 <sup>3</sup> | 1.26        | 7.68                 | 6.07                   |
| <i>PDZRN3</i>           | PDZ domain containing ring finger 3                  | 2.61  | 1.5x10 <sup>2</sup> | 1.39        | 8.00                 | 5.75                   |
| <i>RASSF2</i>           | Ras association (RalGDS/AF-6) domain 2 family member | 3.25  | 1.0x10 <sup>3</sup> | 1.50        | 6.64                 | 4.44                   |
| <i>WNK3</i>             | WNK lysine deficient protein kinase 3                | 3.06  | 5.4x10 <sup>3</sup> | 1.21        | 5.25                 | 4.36                   |
| <i>CDH6</i>             | Cadherin 6, type 2, K-cadherin (fetal kidney)        | 2.80  | 1.1x10 <sup>2</sup> | 1.35        | 5.83                 | 4.31                   |
| <i>CELSR2</i>           | Cadherin, EGF LAG seven-pass G-type receptor 2       | 2.80  | 1.2x10 <sup>2</sup> | 1.21        | 7.78                 | 6.42                   |
| <i>CXCR4</i>            | Chemokine (C-X-C motif) receptor 4                   | 2.55  | 1.5x10 <sup>2</sup> | 1.44        | 6.04                 | 4.20                   |
| <i>CX3CL1</i>           | Chemokine (C-X3-C motif) ligand 1                    | 4.01  | 8.0x10 <sup>4</sup> | 1.26        | 5.89                 | 4.67                   |
| <i>CNIH3</i>            | Cornichon family AMPA receptor auxiliary protein 3   | 2.41  | 3.3x10 <sup>2</sup> | 1.41        | 7.22                 | 5.11                   |
| <i>FABP7</i>            | Fatty acid binding protein 7, brain                  | 2.26  | 3.1x10 <sup>2</sup> | 1.57        | 6.87                 | 4.38                   |
| <i>FBLN1</i>            | Fibulin 1  | 2.62  | 1.9x10 <sup>2</sup> | 1.27        | 7.35                 | 5.78                   |
| <i>FOXA2</i>            | Forkhead box A2                                      | 2.18  | 6.2x10 <sup>2</sup> | 1.36        | 5.56                 | 4.09                   |
| <i>ITPR1</i>            | Inositol 1,4,5-trisphosphate receptor, type 1        | 2.68  | 1.4x10 <sup>2</sup> | 1.25        | 6.99                 | 5.60                   |
| <i>ITPKB</i>            | Inositol-trisphosphate 3-kinase B                    | 2.60  | 1.9x10 <sup>2</sup> | 1.20        | 6.69                 | 5.58                   |
| <i>NRG2</i>             | Neuregulin 2   | 2.73  | 1.4x10 <sup>2</sup> | 1.22        | 4.99                 | 4.09                   |
| <i>NPY1R</i>            | Neuropeptide Y receptor Y1                           | 2.00  | 5.5x10 <sup>2</sup> | 1.41        | 5.73                 | 4.08                   |
| <i>NRP2</i>             | Neuropilin 2   | 2.75  | 1.2x10 <sup>2</sup> | 1.25        | 6.74                 | 5.40                   |

Table V. Continued.

| Gene                        | Description   | Score | P-value             | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|-----------------------------|---|-------|---------------------|-------------|----------------------|------------------------|
| <i>PDE4B</i>                | Phosphodiesterase 4B, cAMP-specific                     | 2.59  | 2.1x10 <sup>2</sup> | 1.26        | 6.83                 | 5.42                   |
| <i>PDGFRL</i>               | Platelet-derived growth factor receptor-like            | 2.55  | 2.5x10 <sup>2</sup> | 1.29        | 6.76                 | 5.22                   |
| <i>SFRP1</i>                | Secreted frizzled-related protein 1                     | 2.33  | 3.5x10 <sup>2</sup> | 1.42        | 7.77                 | 5.46                   |
| <i>SCG2</i>                 | Secretogranin II  | 2.50  | 2.0x10 <sup>2</sup> | 1.43        | 8.08                 | 5.64                   |
| <i>SCUBE3</i>               | Signal peptide, CUB domain, EGF-like 3                  | 2.63  | 3.6x10 <sup>3</sup> | 1.34        | 7.76                 | 5.78                   |
| <i>TLR4</i>                 | Toll-like receptor 4                                    | 2.82  | 9.8x10 <sup>3</sup> | 1.36        | 6.29                 | 4.61                   |
| <i>TMTC1</i>                | Transmembrane and tetratricopeptide repeat containing 1 | 2.43  | 2.5x10 <sup>2</sup> | 1.29        | 6.10                 | 4.72                   |
| Ectoderm development        |   |       |                     |             |                      |                        |
| <i>EPHA3</i>                | EPH receptor A3   | 2.45  | 1.9x10 <sup>2</sup> | 1.39        | 5.33                 | 3.83                   |
| <i>CDH6</i>                 | Cadherin 6, type 2, K-cadherin (fetal kidney)           | 2.80  | 1.1x10 <sup>2</sup> | 1.35        | 5.83                 | 4.31                   |
| <i>CELSR2</i>               | Cadherin, EGF LAG seven-pass G-type receptor 2          | 2.80  | 1.2x10 <sup>2</sup> | 1.21        | 7.78                 | 6.42                   |
| <i>CXCR4</i>                | Chemokine (C-X-C motif) receptor 4                      | 2.55  | 1.5x10 <sup>2</sup> | 1.44        | 6.04                 | 4.20                   |
| <i>FABP7</i>                | Fatty acid binding protein 7, brain                     | 2.26  | 3.1x10 <sup>2</sup> | 1.57        | 6.87                 | 4.38                   |
| <i>FOXA2</i>                | Forkhead box A2   | 2.18  | 6.2x10 <sup>2</sup> | 1.36        | 5.56                 | 4.09                   |
| <i>GPM6B</i>                | Glycoprotein M6B  | 2.14  | 4.8x10 <sup>2</sup> | 1.46        | 7.60                 | 5.19                   |
| <i>HES1</i>                 | Hairy and enhancer of split 1, ( <i>Drosophila</i> )    | 3.29  | 4.2x10 <sup>3</sup> | 1.21        | 8.42                 | 6.98                   |
| <i>HEY1</i>                 | Hairy/enhancer-of-split related with YRPW motif 1       | 2.49  | 2.3x10 <sup>2</sup> | 1.28        | 8.16                 | 6.39                   |
| <i>IRX1</i>                 | Iroquois homeobox 1                                     | 2.81  | 8.4x10 <sup>3</sup> | 1.48        | 6.61                 | 4.47                   |
| <i>JAG1</i>                 | Jagged 1  | 3.16  | 6.0x10 <sup>3</sup> | 1.22        | 7.89                 | 6.48                   |
| <i>NRG2</i>                 | Neuregulin 2  | 2.73  | 1.4x10 <sup>2</sup> | 1.22        | 4.99                 | 4.09                   |
| <i>NRP2</i>                 | Neuropilin 2  | 2.75  | 1.2x10 <sup>2</sup> | 1.25        | 6.74                 | 5.40                   |
| Cell structure and motility |   |       |                     |             |                      |                        |
| <i>CELSR2</i>               | Cadherin, EGF LAG seven-pass G-type receptor 2          | 2.80  | 1.2x10 <sup>2</sup> | 1.21        | 7.78                 | 6.42                   |
| <i>CXCR4</i>                | Chemokine (C-X-C motif) receptor 4                      | 2.55  | 1.5x10 <sup>2</sup> | 1.44        | 6.04                 | 4.20                   |
| <i>COL7A1</i>               | Collagen, type VII, $\alpha$ 1                          | 2.53  | 2.0x10 <sup>2</sup> | 1.26        | 8.29                 | 6.58                   |
| <i>DCLK1</i>                | Doublecortin-like kinase 1                              | 2.69  | 1.6x10 <sup>2</sup> | 1.21        | 4.91                 | 4.06                   |
| <i>DNM3</i>                 | Dynamin 3   | 2.22  | 3.7x10 <sup>2</sup> | 1.21        | 6.13                 | 5.06                   |
| <i>DYNC111</i>              | Dynein, cytoplasmic 1, intermediate chain 1             | 2.85  | 1.1x10 <sup>2</sup> | 1.42        | 7.89                 | 5.55                   |
| <i>FOXA2</i>                | Forkhead box A2   | 2.18  | 6.2x10 <sup>2</sup> | 1.36        | 5.56                 | 4.09                   |
| <i>GPM6B</i>                | Glycoprotein M6B  | 2.14  | 4.8x10 <sup>2</sup> | 1.46        | 7.60                 | 5.19                   |
| <i>ITPR1</i>                | Inositol 1,4,5-trisphosphate receptor, type 1           | 2.68  | 1.4x10 <sup>2</sup> | 1.25        | 6.99                 | 5.60                   |
| <i>JAG1</i>                 | Jagged 1  | 3.16  | 6.0x10 <sup>3</sup> | 1.22        | 7.89                 | 6.48                   |
| <i>MYL5</i>                 | Myosin, light chain 5, regulatory                       | 3.19  | 5.6x10 <sup>3</sup> | 1.25        | 6.73                 | 5.40                   |
| <i>PRICKLE2</i>             | Prickle homolog 2 ( <i>Drosophila</i> )                 | 2.46  | 2.3x10 <sup>2</sup> | 1.22        | 8.15                 | 6.70                   |
| <i>SPP1</i>                 | Secreted phosphoprotein 1                               | 0.82  | 4.2x10 <sup>1</sup> | 1.03        | 7.03                 | 7.22                   |
| Neurogenesis                |   |       |                     |             |                      |                        |
| <i>EPHA3</i>                | EPH receptor A3   | 2.45  | 1.9x10 <sup>2</sup> | 1.39        | 5.33                 | 3.83                   |
| <i>CDH6</i>                 | Cadherin 6, type 2, K-cadherin (fetal kidney)           | 2.80  | 1.1x10 <sup>2</sup> | 1.35        | 5.83                 | 4.31                   |
| <i>CELSR2</i>               | Cadherin, EGF LAG seven-pass G-type receptor 2          | 2.80  | 1.2x10 <sup>2</sup> | 1.21        | 7.78                 | 6.42                   |
| <i>CXCR4</i>                | Chemokine (C-X-C motif) receptor 4                      | 2.55  | 1.5x10 <sup>2</sup> | 1.44        | 6.04                 | 4.20                   |
| <i>FOXA2</i>                | Forkhead box A2   | 2.18  | 6.2x10 <sup>2</sup> | 1.36        | 5.56                 | 4.09                   |
| <i>GPM6B</i>                | Glycoprotein M6B  | 2.14  | 4.8x10 <sup>2</sup> | 1.46        | 7.60                 | 5.19                   |
| <i>HES1</i>                 | Hairy and enhancer of split 1, ( <i>Drosophila</i> )    | 3.29  | 4.2x10 <sup>3</sup> | 1.21        | 8.42                 | 6.98                   |
| <i>HEY1</i>                 | Hairy/enhancer-of-split related with YRPW motif 1       | 2.49  | 2.3x10 <sup>2</sup> | 1.28        | 8.16                 | 6.39                   |
| <i>IRX1</i>                 | Iroquois homeobox 1                                     | 2.81  | 8.4x10 <sup>3</sup> | 1.48        | 6.61                 | 4.47                   |
| <i>JAG1</i>                 | Jagged 1  | 3.16  | 6.0x10 <sup>3</sup> | 1.22        | 7.89                 | 6.48                   |
| <i>NRG2</i>                 | Neuregulin 2  | 2.73  | 1.4x10 <sup>2</sup> | 1.22        | 4.99                 | 4.09                   |
| <i>NRP2</i>                 | Neuropilin 2  | 2.75  | 1.2x10 <sup>2</sup> | 1.25        | 6.74                 | 5.40                   |



Table V. Continued.

| Gene                       | Description   | Score | P-value             | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|----------------------------|---|-------|---------------------|-------------|----------------------|------------------------|
| Cell communication         |   |       |                     |             |                      |                        |
| <i>CDH6</i>                | Cadherin 6, type 2, K-cadherin (fetal kidney)                                 | 2.80  | 1.1x10 <sup>2</sup> | 1.35        | 5.83                 | 4.31                   |
| <i>CELSR2</i>              | Cadherin, EGF LAG seven-pass G-type receptor 2                                | 2.80  | 1.2x10 <sup>2</sup> | 1.21        | 7.78                 | 6.42                   |
| <i>FABP7</i>               | Fatty acid binding protein 7, brain   | 2.26  | 3.1x10 <sup>2</sup> | 1.57        | 6.87                 | 4.38                   |
| <i>FBLN1</i>               | Fibulin 1   | 2.62  | 1.9x10 <sup>2</sup> | 1.27        | 7.35                 | 5.78                   |
| <i>FOXA2</i>               | Forkhead box A2   | 2.18  | 6.2x10 <sup>2</sup> | 1.36        | 5.56                 | 4.09                   |
| <i>ITPR1</i>               | Inositol 1,4,5-trisphosphate receptor, type 1                                 | 2.68  | 1.4x10 <sup>2</sup> | 1.25        | 6.99                 | 5.60                   |
| <i>NRG2</i>                | Neuregulin 2  | 2.73  | 1.4x10 <sup>2</sup> | 1.22        | 4.99                 | 4.09                   |
| <i>SFRP1</i>               | Secreted frizzled-related protein 1   | 2.33  | 3.5x10 <sup>2</sup> | 1.42        | 7.77                 | 5.46                   |
| <i>SCG2</i>                | Secretogranin II  | 2.50  | 2.0x10 <sup>2</sup> | 1.43        | 8.08                 | 5.64                   |
| <i>SCUBE3</i>              | Signal peptide, CUB domain, EGF-like 3  | 2.63  | 3.6x10 <sup>3</sup> | 1.34        | 7.76                 | 5.78                   |
| <i>TMTC1</i>               | Transmembrane and tetratricopeptide repeat containing 1                       | 2.43  | 2.5x10 <sup>2</sup> | 1.29        | 6.10                 | 4.72                   |
| Mesoderm development       |   |       |                     |             |                      |                        |
| <i>EFHD1</i>               | EF-hand domain family, member D1  | 2.22  | 4.4x10 <sup>2</sup> | 1.31        | 6.03                 | 4.61                   |
| <i>EPHA3</i>               | EPH receptor A3   | 2.45  | 1.9x10 <sup>2</sup> | 1.39        | 5.33                 | 3.83                   |
| <i>FBLN1</i>               | Fibulin 1   | 2.62  | 1.9x10 <sup>2</sup> | 1.27        | 7.35                 | 5.78                   |
| <i>FOXA2</i>               | Forkhead box A2   | 2.18  | 6.2x10 <sup>2</sup> | 1.36        | 5.56                 | 4.09                   |
| <i>MYL5</i>                | Myosin, light chain 5, regulatory   | 3.19  | 5.6x10 <sup>3</sup> | 1.25        | 6.73                 | 5.40                   |
| <i>NRP2</i>                | Neuropilin 2  | 2.75  | 1.2x10 <sup>2</sup> | 1.25        | 6.74                 | 5.40                   |
| <i>PTHLH</i>               | Parathyroid hormone-like hormone  | 2.46  | 1.9x10 <sup>2</sup> | 1.42        | 6.77                 | 4.75                   |
| <i>SCUBE3</i>              | Signal peptide, CUB domain, EGF-like 3  | 2.63  | 3.6x10 <sup>3</sup> | 1.34        | 7.76                 | 5.78                   |
| Cell structure             |   |       |                     |             |                      |                        |
| <i>CELSR2</i>              | Cadherin, EGF LAG seven-pass G-type receptor 2                                | 2.80  | 1.2x10 <sup>2</sup> | 1.21        | 7.78                 | 6.42                   |
| <i>COL7A1</i>              | Collagen, type VII, $\alpha$ 1  | 2.53  | 2.0x10 <sup>2</sup> | 1.26        | 8.29                 | 6.58                   |
| <i>DCLK1</i>               | Doublecortin-like kinase 1  | 2.69  | 1.6x10 <sup>2</sup> | 1.21        | 4.91                 | 4.06                   |
| <i>DNM3</i>                | Dynamin 3   | 2.22  | 3.7x10 <sup>2</sup> | 1.21        | 6.13                 | 5.06                   |
| <i>DYNC111</i>             | Dynein, cytoplasmic 1, intermediate chain 1                                   | 2.85  | 1.1x10 <sup>2</sup> | 1.42        | 7.89                 | 5.55                   |
| <i>FOXA2</i>               | Forkhead box A2   | 2.18  | 6.2x10 <sup>2</sup> | 1.36        | 5.56                 | 4.09                   |
| <i>GPM6B</i>               | Glycoprotein M6B  | 2.14  | 4.8x10 <sup>2</sup> | 1.46        | 7.60                 | 5.19                   |
| <i>SPP1</i>                | Secreted phosphoprotein 1   | 0.82  | 4.2x10 <sup>1</sup> | 1.03        | 7.03                 | 7.22                   |
| Unknown biological process |   |       |                     |             |                      |                        |
| <i>RNF182</i>              | Ring finger protein 182   | 2.22  | 3.9x10 <sup>2</sup> | 1.27        | 8.41                 | 6.64                   |
| <i>ACSS3</i>               | Acyl-CoA synthetase short-chain family member 3                               | 2.48  | 3.3x10 <sup>2</sup> | 1.28        | 6.52                 | 5.08                   |
| <i>GSTM4</i>               | Glutathione S-transferase mu 4  | 4.79  | 4.0x10 <sup>4</sup> | 1.41        | 7.93                 | 5.62                   |
| <i>LINC00461</i>           | Long intergenic non-protein coding RNA 461                                    | 4.67  | 6.0x10 <sup>4</sup> | 1.55        | 9.31                 | 5.99                   |
| <i>FAM70A</i>              | Transmembrane protein 255A  | 3.80  | 6.0x10 <sup>4</sup> | 1.72        | 7.46                 | 4.33                   |
| <i>COL21A1</i>             | Collagen, type XXI, $\alpha$ 1  | 4.49  | 4.0x10 <sup>4</sup> | 1.74        | 7.61                 | 4.38                   |
| <i>METTL7A</i>             | Methyltransferase like 7A   | 3.32  | 5.0x10 <sup>3</sup> | 1.49        | 8.06                 | 5.40                   |
| <i>GMPR</i>                | Guanosine monophosphate reductase   | 0.33  | 7.5x10 <sup>1</sup> | 1.01        | 8.81                 | 8.94                   |
| <i>NID1</i>                | Nidogen 1   | 2.36  | 2.8x10 <sup>2</sup> | 1.26        | 9.12                 | 7.23                   |
| <i>KIAA0895</i>            | KIAA0895  | 2.04  | 5.5x10 <sup>2</sup> | 1.21        | 6.57                 | 5.44                   |
| <i>C8orf4</i>              | Chromosome 8 open reading frame 4   | 0.91  | 3.7x10 <sup>1</sup> | 1.04        | 10.02                | 9.67                   |
| <i>SEL1L3</i>              | Sel-1 suppressor of lin-12-like 3 ( <i>Caenorhabditis elegans</i> )           | 2.19  | 4.3x10 <sup>2</sup> | 1.33        | 8.99                 | 6.76                   |
| <i>GPC4</i>                | Glypican 4  | 2.55  | 2.2x10 <sup>2</sup> | 1.41        | 8.55                 | 6.07                   |
| <i>PLEKHG1</i>             | Pleckstrin homology domain containing, family G (with RhoGef domain) member 1 | 2.47  | 2.8x10 <sup>2</sup> | 1.38        | 6.36                 | 4.62                   |

Table V. Continued.

| Gene            | Description   | Score | P-value             | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|-----------------|---|-------|---------------------|-------------|----------------------|------------------------|
| <i>PIPOX</i>    | Pipecolic acid oxidase  | 3.29  | 4.0x10 <sup>4</sup> | 1.68        | 6.46                 | 3.84                   |
| <i>FAM65B</i>   | Family with sequence similarity 65, member B                              | 2.56  | 1.1x10 <sup>2</sup> | 1.39        | 5.57                 | 3.99                   |
| <i>C7orf57</i>  | Chromosome 7 open reading frame 57  | 2.17  | 4.2x10 <sup>2</sup> | 1.46        | 5.56                 | 3.80                   |
| <i>PPP2R2B</i>  | Protein phosphatase 2, regulatory subunit B, $\beta$                      | 3.58  | 2.8x10 <sup>3</sup> | 1.61        | 7.44                 | 4.62                   |
| <i>SERP2</i>    | Stress-associated endoplasmic reticulum protein family member 2           | 2.11  | 5.2x10 <sup>2</sup> | 1.22        | 6.19                 | 5.09                   |
| <i>SOX2</i>     | SRY (sex determining region Y)-box 2                                      | 1.23  | 2.5x10 <sup>1</sup> | 1.04        | 4.07                 | 3.92                   |
| <i>RPRM</i>     | Reprimo, TP53 dependent G2 arrest mediator candidate                      | 0.43  | 6.9x10 <sup>1</sup> | 1.01        | 3.99                 | 4.04                   |
| <i>MFSD2A</i>   | Major facilitator superfamily domain containing 2A                        | 3.69  | 2.0x10 <sup>3</sup> | 1.30        | 7.33                 | 5.63                   |
| <i>PELI2</i>    | Pellino E3 ubiquitin protein ligase family member 2                       | 2.91  | 1.1x10 <sup>2</sup> | 1.29        | 7.33                 | 5.68                   |
| <i>GCNT2</i>    | Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group) | 2.40  | 3.3x10 <sup>2</sup> | 1.22        | 7.59                 | 6.22                   |
| <i>SLC16A4</i>  | Solute carrier family 16, member 4  | 2.88  | 1.1x10 <sup>2</sup> | 1.39        | 8.00                 | 5.77                   |
| <i>SH3BGR</i>   | SH3 domain binding glutamic acid-rich protein                             | 1.58  | 1.3x10 <sup>1</sup> | 1.05        | 10.64                | 10.12                  |
| <i>WDR31</i>    | WD repeat domain 31   | 3.54  | 2.8x10 <sup>3</sup> | 1.20        | 5.83                 | 4.86                   |
| <i>SLC16A9</i>  | Solute carrier family 16, member 9  | 2.07  | 4.4x10 <sup>2</sup> | 1.23        | 6.40                 | 5.19                   |
| <i>GSTT1</i>    | Glutathione S-transferase theta 1   | 2.91  | 1.3x10 <sup>2</sup> | 1.40        | 7.41                 | 5.31                   |
| <i>NDP</i>      | Norrie disease (pseudoglioma)   | 2.53  | 2.4x10 <sup>2</sup> | 1.50        | 7.62                 | 5.09                   |
| <i>NDN</i>      | Necdin, melanoma antigen (MAGE) family member                             | 2.42  | 2.9x10 <sup>2</sup> | 1.44        | 7.59                 | 5.27                   |
| <i>ASB9</i>     | Ankyrin repeat and SOCS box containing 9                                  | 2.20  | 4.3x10 <sup>2</sup> | 1.26        | 7.03                 | 5.58                   |
| <i>LONRF2</i>   | LON peptidase N-terminal domain and ring finger 2                         | 2.08  | 6.0x10 <sup>2</sup> | 1.37        | 6.10                 | 4.44                   |
| <i>SPHAR</i>    | S-phase response (cyclin related)   | 2.62  | 1.8x10 <sup>2</sup> | 1.22        | 7.49                 | 6.12                   |
| <i>RNF144A</i>  | Ring finger protein 144A  | 2.62  | 1.6x10 <sup>2</sup> | 1.24        | 7.07                 | 5.71                   |
| <i>SERINC5</i>  | Serine incorporator 5   | 4.07  | 1.4x10 <sup>3</sup> | 1.20        | 10.73                | 8.95                   |
| <i>RRAGD</i>    | Ras-related GTP binding D   | 2.42  | 3.0x10 <sup>2</sup> | 1.28        | 8.29                 | 6.48                   |
| <i>OGDHL</i>    | Oxoglutarate dehydrogenase-like   | 2.65  | 1.5x10 <sup>2</sup> | 1.25        | 6.36                 | 5.11                   |
| <i>CEND1</i>    | Cell cycle exit and neuronal differentiation 1                            | 3.91  | 1.0x10 <sup>3</sup> | 1.24        | 6.38                 | 5.14                   |
| <i>RBPM52</i>   | RNA binding protein with multiple splicing 2                              | 2.11  | 4.6x10 <sup>2</sup> | 1.26        | 6.34                 | 5.03                   |
| <i>SULF2</i>    | Sulfatase 2   | 2.69  | 1.9x10 <sup>2</sup> | 1.50        | 8.01                 | 5.33                   |
| <i>MMP7</i>     | Matrix metalloproteinase 7 (matrilysin, uterine)                          | 2.97  | 2.0x10 <sup>3</sup> | 1.24        | 5.14                 | 4.15                   |
| <i>SLC2A12</i>  | Solute carrier family 2 (facilitated glucose transporter), member 12      | 2.95  | 8.4x10 <sup>3</sup> | 1.35        | 6.31                 | 4.67                   |
| <i>GFPT2</i>    | Glutamine-fructose-6-phosphate transaminase 2                             | 2.24  | 3.7x10 <sup>2</sup> | 1.29        | 8.35                 | 6.46                   |
| <i>SOX9</i>     | SRY (sex determining region Y)-box 9                                      | 2.18  | 4.3x10 <sup>2</sup> | 1.31        | 9.42                 | 7.17                   |
| <i>C5orf46</i>  | Chromosome 5 open reading frame 46  | 2.29  | 3.2x10 <sup>2</sup> | 1.34        | 8.92                 | 6.67                   |
| <i>CP</i>       | Ceruloplasmin (ferroxidase)   | 2.35  | 3.3x10 <sup>2</sup> | 1.05        | 4.24                 | 4.03                   |
| <i>GPNMB</i>    | Glycoprotein (transmembrane) nmb  | 2.85  | 1.1x10 <sup>2</sup> | 1.35        | 10.04                | 7.46                   |
| <i>SERPINI1</i> | Serpin peptidase inhibitor, clade I (neuroserpin), member 1               | 2.35  | 3.5x10 <sup>2</sup> | 1.32        | 7.42                 | 5.63                   |
| <i>TPRG1</i>    | Tumor protein p63 regulated 1   | 2.36  | 3.5x10 <sup>2</sup> | 1.30        | 5.12                 | 3.94                   |
| <i>PITX2</i>    | Paired-like homeodomain 2   | 2.09  | 5.6x10 <sup>2</sup> | 1.32        | 5.44                 | 4.13                   |

<sup>a</sup>Up, and down mean refers to the mean of the specific gene expression levels in the ten most PGC1 $\alpha$  up- or downregulated cell lines.

with a previous study that detected a brain-specific isoform of PGC1 $\alpha$  in the cytoplasm rather than the nucleus (27). It was also reported that the PGC1 $\alpha$  isoform becomes localized in the mitochondria via phosphatase and tensin

homolog-induced putative kinase 1 and voltage-dependent anion channel (28).

This present study also demonstrated that PGC1 $\alpha$  was expressed in the mitochondria of GBM cells. Based on these

Table VI. Annotated summary of class A of peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$ .

| Functional role                    | Genes | P-value            | -Log (P-value) |
|------------------------------------|-------|--------------------|----------------|
| Biological process                 |       |                    |                |
| Developmental processes            | 28    | $4.30 \times 10^6$ | 5.37           |
| Ectoderm development               | 13    | $2.10 \times 10^4$ | 3.68           |
| Neurogenesis                       | 12    | $2.50 \times 10^4$ | 3.60           |
| Cell structure and motility        | 13    | $1.20 \times 10^2$ | 1.92           |
| Mesoderm development               | 8     | $2.70 \times 10^2$ | 1.57           |
| Cell structure                     | 8     | $5.80 \times 10^2$ | 1.24           |
| Signal transduction                | 25    | $6.60 \times 10^2$ | 1.18           |
| Cell communication                 | 11    | $9.40 \times 10^2$ | 1.03           |
| Cellular component                 |       |                    |                |
| Extracellular region part          | 16    | $1.30 \times 10^4$ | 3.89           |
| Extracellular region               | 23    | $5.70 \times 10^4$ | 3.24           |
| Extracellular matrix               | 8     | $2.30 \times 10^3$ | 2.64           |
| Extracellular space                | 11    | $3.20 \times 10^3$ | 2.49           |
| Proteinaceous extracellular matrix | 7     | $6.90 \times 10^3$ | 2.16           |

The dataset of significantly changed genes were identified using the Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov>) ( $P < 0.05$ ).

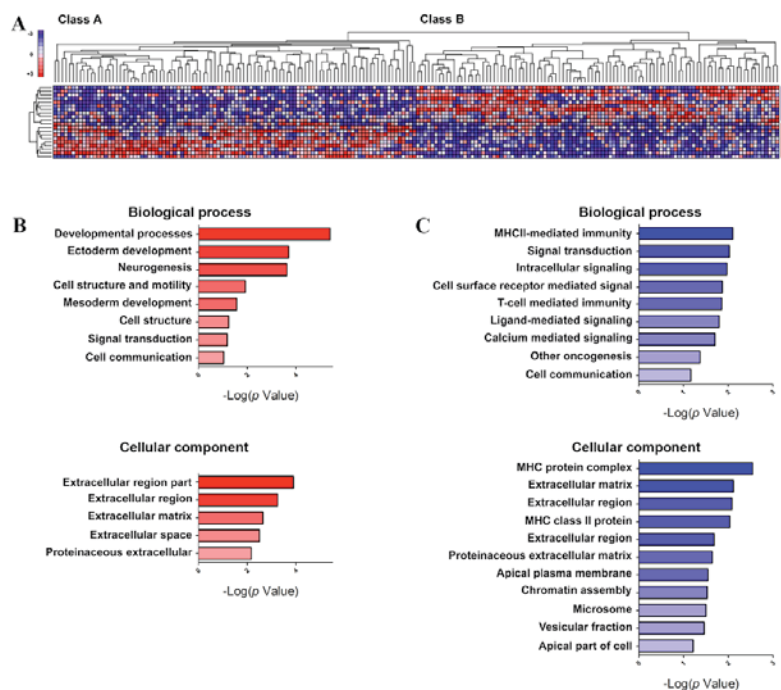


Figure 5. Bioinformatics analysis of *PGC1 $\alpha$* -associated genes in two classes of GBM cell lines. (A) Two-way hierarchical clustering of differentially expressed genes in the top ten *PGC1 $\alpha$*  up- and downregulated GBM cell lines by Pearson distance. (B) Class A genes were divided into biological processes, molecular functions or cellular components. (C) Genes in class B were sorted by biological process, molecular function and cellular component. Color in the heat-maps displays expression relative to the mean expression value, with red indicating higher expression and blue lower expression. GBM, glioblastoma multiforme' *PGC1 $\alpha$* , peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$ .

corroborating results, it is predicted that *PGC1 $\alpha$* -mediated mitochondrial biogenesis and respiration is increased in GBM cells.

To investigate the role *PGC1 $\alpha$*  has in GBM cells, several bioinformatics analyses were performed. The analyses

demonstrated that metabolic and mitochondrial genes were highly correlated with *PGC1 $\alpha$*  in a number of GBM cell lines. Class Neighbors analysis classified *PGC1 $\alpha$* -expressing GBM cell lines into two groups: Class A and B. Class A contained genes associated with development, neurogenesis, cell structure

Table VII. List of class B genes highly expressed in peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  downregulated glioblastoma multiforme cells.

| Gene   | Description   | Score | P-value              | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|--|---|-------|----------------------|-------------|----------------------|------------------------|
| Major histocompatibility complex, class II-mediated immunity |   |       |                      |             |                      |                        |
| <i>HLA-DMA</i>   | Major histocompatibility complex, class II, DM $\alpha$   | 2.32  | 3.4x10 <sup>2</sup>  | 1.34        | 5.69                 | 7.66                   |
| <i>HLA-DRB1</i>  | Major histocompatibility complex, class II, DR $\beta$ 1  | 2.18  | 4.5x10 <sup>2</sup>  | 1.35        | 5.99                 | 8.08                   |
| <i>HLA-DQB1</i>  | Major histocompatibility complex, class II, DQ $\beta$ 1  | 2.22  | 3.6x10 <sup>2</sup>  | 1.26        | 5.16                 | 6.49                   |
| Signal transduction  |   |       |                      |             |                      |                        |
| <i>ADAMTS1</i>   | ADAM metalloproteinase with thrombospondin type 1 motif, 1  | 1.16  | 1.2x10 <sup>1</sup>  | 1.10        | 3.49                 | 3.83                   |
| <i>ADAMTS6</i>   | ADAM metalloproteinase with thrombospondin type 1 motif, 6  | 2.16  | 2.1x10 <sup>2</sup>  | 1.31        | 4.71                 | 6.17                   |
| <i>ARAP2</i>   | ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2   | 2.16  | 4.9x10 <sup>2</sup>  | 1.27        | 4.51                 | 5.74                   |
| <i>BAIAP2L1</i>  | BAI1-associated protein 2-like 1  | 2.13  | 5.1x10 <sup>2</sup>  | 1.22        | 5.84                 | 7.10                   |
| <i>CD33</i>  | CD33 molecule   | 2.54  | 6.6x10 <sup>3</sup>  | 1.24        | 4.54                 | 5.64                   |
| <i>DEPDC7</i>  | DEP domain containing 7   | 2.13  | 5.0x10 <sup>2</sup>  | 1.23        | 6.86                 | 8.47                   |
| <i>FCRLB</i>   | Fc receptor-like B  | 2.89  | 1.3x10 <sup>2</sup>  | 1.23        | 5.38                 | 6.60                   |
| <i>RAB3B</i>   | RAB3B, member RAS oncogene family   | 2.75  | 1.1x10 <sup>2</sup>  | 1.39        | 4.80                 | 6.68                   |
| <i>SLITRK5</i>   | SLIT and NTRK-like family, member 5   | 2.59  | 1.6x10 <sup>2</sup>  | 1.29        | 5.21                 | 6.70                   |
| <i>ADRB2</i>   | Adrenoceptor $\beta$ 2, surface   | 3.28  | 4.2x10 <sup>3</sup>  | 1.34        | 5.85                 | 7.85                   |
| <i>AHRR</i>  | Aryl-hydrocarbon receptor repressor   | 2.06  | 5.5x10 <sup>2</sup>  | 1.25        | 6.24                 | 7.83                   |
| <i>CALB2</i>   | Calbindin 2   | 2.46  | 1.7x10 <sup>2</sup>  | 1.36        | 4.57                 | 6.23                   |
| <i>F2RL2</i>   | Coagulation factor II (thrombin) receptor-like 2  | 2.24  | 3.4x10 <sup>2</sup>  | 1.39        | 4.33                 | 6.04                   |
| <i>FGF1</i>  | Fibroblast growth factor 1 (acidic)   | 2.06  | 5.0x10 <sup>2</sup>  | 1.31        | 4.35                 | 5.69                   |
| <i>GRB14</i>   | Growth factor receptor-bound protein 14   | 2.08  | 4.8x10 <sup>2</sup>  | 1.25        | 4.24                 | 5.29                   |
| <i>IL12A</i>   | Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte Maturation factor 1, p35) | 3.58  | 1.8x10 <sup>3</sup>  | 1.26        | 4.24                 | 5.35                   |
| <i>IL4R</i>  | Interleukin 4 receptor  | 2.50  | 1.7x10 <sup>2</sup>  | 1.21        | 5.42                 | 6.54                   |
| <i>OR51B4</i>  | Olfactory receptor, family 51, subfamily B, member 4  | 2.43  | 6.0x10 <sup>3</sup>  | 1.23        | 4.25                 | 5.22                   |
| <i>OXTN</i>  | Oxytocin receptor   | 2.29  | 2.8x10 <sup>2</sup>  | 1.31        | 5.90                 | 7.70                   |
| <i>PLCB4</i>   | Phospholipase C, $\beta$ 4  | 2.66  | 1.9x10 <sup>2</sup>  | 1.31        | 6.48                 | 8.50                   |
| <i>PDGFA</i>   | Platelet-derived growth factor $\alpha$ polypeptide   | 2.29  | 3.6x10 <sup>2</sup>  | 1.26        | 6.68                 | 8.43                   |
| <i>PTPN22</i>  | Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)   | 2.79  | 1.3x10 <sup>2</sup>  | 1.29        | 3.60                 | 4.65                   |
| <i>RGS10</i>   | Regulator of G-protein signaling 10   | 2.96  | 5.8x10 <sup>3</sup>  | 1.19        | 8.25                 | 9.83                   |
| <i>STYK1</i>   | Serine/threonine/tyrosine kinase 1  | 2.25  | 1.9x10 <sup>2</sup>  | 1.25        | 4.15                 | 5.17                   |
| <i>SPHK1</i>   | Sphingosine kinase 1  | 2.05  | 5.2x10 <sup>2</sup>  | 1.20        | 6.57                 | 7.86                   |
| <i>STC2</i>  | Stanniocalcin 2   | 2.12  | 4.9x10 <sup>2</sup>  | 1.23        | 7.08                 | 8.68                   |
| <i>WNT5B</i>   | Wingless-type MMTV integration site family, member 5B   | 3.11  | 7.4x10 <sup>3</sup>  | 1.32        | 5.19                 | 6.84                   |
| Intracellular signaling cascade                              |   |       |                      |             |                      |                        |
| <i>DEPDC7</i>  | DEP domain containing 7   | 2.13  | 5.0 x10 <sup>2</sup> | 1.23        | 6.86                 | 8.47                   |
| <i>RAB3B</i>   | RAB3B, member RAS oncogene family   | 2.75  | 1.1x10 <sup>2</sup>  | 1.39        | 4.80                 | 6.68                   |
| <i>ADRB2</i>   | Adrenoceptor $\beta$ 2, surface   | 3.28  | 4.2x10 <sup>3</sup>  | 1.34        | 5.85                 | 7.85                   |
| <i>AHRR</i>  | Aryl-hydrocarbon receptor repressor   | 2.06  | 5.5x10 <sup>2</sup>  | 1.25        | 6.24                 | 7.83                   |
| <i>CALB2</i>   | Calbindin 2   | 2.46  | 1.7x10 <sup>2</sup>  | 1.36        | 4.57                 | 6.23                   |
| <i>FGF1</i>  | Fibroblast growth factor 1 (acidic)   | 2.06  | 5.0x10 <sup>2</sup>  | 1.31        | 4.35                 | 5.69                   |



Table VII. Continued.

| Gene   | Description   | Score | P-value               | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|--|---|-------|-----------------------|-------------|----------------------|------------------------|
| <i>IL12A</i>                                       | Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte Maturation factor 1, p35) | 3.58  | 1.8x10 <sup>-3</sup>  | 1.26        | 4.24                 | 5.35                   |
| <i>IL4R</i>  | Interleukin 4 receptor  | 2.50  | 1.7x10 <sup>-2</sup>  | 1.21        | 5.42                 | 6.54                   |
| <i>OXTR</i>  | Oxytocin receptor   | 2.29  | 2.8x10 <sup>-2</sup>  | 1.31        | 5.90                 | 7.70                   |
| <i>PLCB4</i>                                       | Phospholipase C, $\beta$ 4  | 2.66  | 1.9x10 <sup>-2</sup>  | 1.31        | 6.48                 | 8.50                   |
| <i>PDGFA</i>                                       | Platelet-derived growth factor $\alpha$ polypeptide   | 2.29  | 3.6x10 <sup>-2</sup>  | 1.26        | 6.68                 | 8.43                   |
| Cell surface receptor mediated signal transduction |   |       |                       |             |                      |                        |
| <i>ARAP2</i>                                       | ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2   | 2.16  | 4.9x10 <sup>-2</sup>  | 1.27        | 4.51                 | 5.74                   |
| <i>CD33</i>  | CD33 molecule   | 2.54  | 6.6x10 <sup>-3</sup>  | 1.24        | 4.54                 | 5.64                   |
| <i>SLITRK5</i>                                     | SLIT and NTRK-like family, member 5   | 2.59  | 1.6x10 <sup>-2</sup>  | 1.29        | 5.21                 | 6.70                   |
| <i>ADRB2</i>                                       | Adrenoceptor $\beta$ 2, surface   | 3.28  | 4.2x10 <sup>-3</sup>  | 1.34        | 5.85                 | 7.85                   |
| <i>F2RL2</i>                                       | Coagulation factor II (thrombin) receptor-like 2  | 2.24  | 3.4x10 <sup>-2</sup>  | 1.39        | 4.33                 | 6.04                   |
| <i>FGF1</i>  | Fibroblast growth factor 1 (acidic)   | 2.06  | 5.0x10 <sup>-2</sup>  | 1.31        | 4.35                 | 5.69                   |
| <i>GRB14</i>                                       | Growth factor receptor-bound protein 14   | 2.08  | 4.8x10 <sup>-2</sup>  | 1.25        | 4.24                 | 5.29                   |
| <i>IL12A</i>                                       | Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35) | 3.58  | 1.8x10 <sup>-3</sup>  | 1.26        | 4.24                 | 5.35                   |
| <i>IL4R</i>  | Interleukin 4 receptor  | 2.50  | 1.7x10 <sup>-2</sup>  | 1.21        | 5.42                 | 6.54                   |
| <i>OR51B4</i>                                      | Olfactory receptor, family 51, subfamily B, member 4  | 2.43  | 6.0x10 <sup>-3</sup>  | 1.23        | 4.25                 | 5.22                   |
| <i>OXTR</i>  | Oxytocin receptor   | 2.29  | 2.8x10 <sup>-2</sup>  | 1.31        | 5.90                 | 7.70                   |
| <i>PDGFA</i>                                       | Platelet-derived growth factor $\alpha$ polypeptide   | 2.29  | 3.6x10 <sup>-2</sup>  | 1.26        | 6.68                 | 8.43                   |
| <i>PTPN22</i>                                      | Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)   | 2.79  | 1.3x10 <sup>-2</sup>  | 1.29        | 3.60                 | 4.65                   |
| <i>RGS10</i>                                       | Regulator of G-protein signaling 10   | 2.96  | 5.8x10 <sup>-3</sup>  | 1.19        | 8.25                 | 9.83                   |
| <i>STYK1</i>                                       | Serine/threonine/tyrosine kinase 1  | 2.25  | 1.9x10 <sup>-2</sup>  | 1.25        | 4.15                 | 5.17                   |
| <i>STC2</i>  | Stanniocalcin 2   | 2.12  | 4.9x10 <sup>-2</sup>  | 1.23        | 7.08                 | 8.68                   |
| T-cell mediated immunity                           |   |       |                       |             |                      |                        |
| <i>FOSL1</i>                                       | FOS-like antigen 1  | 2.36  | 3.2x10 <sup>-2</sup>  | 1.25        | 7.99                 | 9.99                   |
| <i>IL12A</i>                                       | Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35) | 3.58  | 1.8x10 <sup>-3</sup>  | 1.26        | 4.24                 | 5.35                   |
| <i>HLA-DMA</i>                                     | Major histocompatibility complex, class II, DM $\alpha$   | 2.32  | 3.4x10 <sup>-2</sup>  | 1.34        | 5.69                 | 7.66                   |
| <i>HLA-DRB1</i>                                    | Major histocompatibility complex, class II, DR $\beta$ 1  | 2.18  | 4.5x10 <sup>-2</sup>  | 1.35        | 5.99                 | 8.08                   |
| <i>HLA-DQB1</i>                                    | Major histocompatibility complex, class II, DQ $\beta$ 1  | 2.22  | 3.6x10 <sup>-2</sup>  | 1.26        | 5.16                 | 6.49                   |
| Ligand-mediated signaling                          |   |       |                       |             |                      |                        |
| <i>ADRB2</i>                                       | Adrenoceptor $\beta$ 2, surface   | 3.28  | 4.2 x10 <sup>-3</sup> | 1.34        | 5.85                 | 7.85                   |
| <i>AHRR</i>  | Aryl-hydrocarbon receptor repressor   | 2.06  | 5.5 x10 <sup>-2</sup> | 1.25        | 6.24                 | 7.83                   |
| <i>FGF1</i>  | Fibroblast growth factor 1 (acidic)   | 1.37  | 1.9x10 <sup>-1</sup>  | 1.03        | 3.89                 | 4.00                   |
| <i>IL12A</i>                                       | Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte Maturation factor 1, p35) | 3.58  | 1.8x10 <sup>-3</sup>  | 1.26        | 4.24                 | 5.35                   |
| <i>IL4R</i>  | Interleukin 4 receptor  | 2.50  | 1.7x10 <sup>-2</sup>  | 1.21        | 5.42                 | 6.54                   |
| <i>PDGFA</i>                                       | Platelet-derived growth factor $\alpha$ polypeptide   | 2.29  | 3.6x10 <sup>-2</sup>  | 1.26        | 6.68                 | 8.43                   |
| <i>WNT5B</i>                                       | Wingless-type MMTV integration site family, member 5B   | 3.11  | 7.4x10 <sup>-3</sup>  | 1.32        | 5.19                 | 6.84                   |

Table VII. Continued.

| Gene                       | Description  | Score | P-value             | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|----------------------------|--|-------|---------------------|-------------|----------------------|------------------------|
| Calcium mediated signaling |  |       |                     |             |                      |                        |
| <i>ADRB2</i>               | Adrenoceptor $\beta$ 2, surface  | 3.28  | 4.2x10 <sup>3</sup> | 1.34        | 5.85                 | 7.85                   |
| <i>CALB2</i>               | Calbindin 2  | 2.46  | 1.7x10 <sup>2</sup> | 1.36        | 4.57                 | 6.23                   |
| <i>OXTR</i>                | Oxytocin receptor  | 2.29  | 2.8x10 <sup>2</sup> | 1.31        | 5.90                 | 7.70                   |
| <i>PDGFA</i>               | Platelet-derived growth factor $\alpha$ polypeptide  | 2.29  | 3.6x10 <sup>2</sup> | 1.26        | 6.68                 | 8.43                   |
| Oncogenesis-associated     |  |       |                     |             |                      |                        |
| <i>MAGEA1</i>              | Melanoma antigen family A, 1 (directs expression of antigen MZ2-E)   | 1.55  | 1.3x10 <sup>1</sup> | 1.36        | 4.60                 | 6.23                   |
| <i>MAGEA11</i>             | Melanoma antigen family A, 11  | 2.88  | 1.2x10 <sup>2</sup> | 1.72        | 3.70                 | 6.37                   |
| <i>MAGEC2</i>              | Melanoma antigen family C, 2   | 2.06  | 4.3x10 <sup>2</sup> | 1.35        | 5.44                 | 7.34                   |
| Cell communication         |  |       |                     |             |                      |                        |
| <i>ADAMTS1</i>             | ADAM metalloproteinase with thrombospondin type 1 motif, 1   | 1.16  | 1.2x10 <sup>1</sup> | 1.10        | 3.49                 | 3.83                   |
| <i>ADAMTS6</i>             | ADAM metalloproteinase with thrombospondin type 1 motif, 6   | 2.16  | 2.1x10 <sup>2</sup> | 1.31        | 4.71                 | 6.17                   |
| <i>CD33</i>                | CD33 molecule  | 2.54  | 6.6x10 <sup>3</sup> | 1.24        | 4.54                 | 5.64                   |
| <i>ADRB2</i>               | Adrenoceptor $\beta$ 2, surface  | 3.28  | 4.2x10 <sup>3</sup> | 1.34        | 5.85                 | 7.85                   |
| <i>AHRR</i>                | Aryl-hydrocarbon receptor repressor  | 2.06  | 5.5x10 <sup>2</sup> | 1.25        | 6.24                 | 7.83                   |
| <i>FGF1</i>                | Fibroblast growth factor 1 (acidic)  | 1.37  | 1.9x10 <sup>1</sup> | 1.03        | 3.89                 | 4.00                   |
| <i>IL12A</i>               | Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte Maturation factor 1, p35)                  | 3.58  | 1.8x10 <sup>3</sup> | 1.26        | 4.24                 | 5.35                   |
| <i>IL4R</i>                | Interleukin 4 receptor   | 2.50  | 1.7x10 <sup>2</sup> | 1.21        | 5.42                 | 6.54                   |
| <i>PDGFA</i>               | Platelet-derived growth factor $\alpha$ polypeptide  | 2.29  | 3.6x10 <sup>2</sup> | 1.26        | 6.68                 | 8.43                   |
| <i>PTPN22</i>              | Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)  | 2.79  | 1.3x10 <sup>2</sup> | 1.29        | 3.60                 | 4.65                   |
| <i>WNT5B</i>               | Wingless-type MMTV integration site family, member 5B  | 3.11  | 7.4x10 <sup>3</sup> | 1.32        | 5.19                 | 6.84                   |
| Unknown biological process |  |       |                     |             |                      |                        |
| <i>FST</i>                 | Follistatin  | 2.56  | 2.2x10 <sup>2</sup> | 1.36        | 6.21                 | 8.43                   |
| <i>SMTN</i>                | Smoothelin   | 1.99  | 6.6x10 <sup>2</sup> | 1.03        | 3.75                 | 3.63                   |
| <i>AOX1</i>                | Aldehyde oxidase 1   | 4.61  | 2.0x10 <sup>4</sup> | 1.59        | 4.65                 | 7.38                   |
| <i>SH2D5</i>               | SH2 domain containing 5  | 3.37  | 1.8x10 <sup>3</sup> | 1.26        | 4.95                 | 6.24                   |
| <i>KIAA1609</i>            | TBC/LysM-associated domain containing 1  | 4.08  | 6.0x10 <sup>4</sup> | 1.26        | 5.66                 | 7.14                   |
| <i>VEPH1</i>               | Ventricular zone expressed PH domain-containing 1  | 2.10  | 4.4x10 <sup>2</sup> | 1.24        | 5.03                 | 6.24                   |
| <i>MEOX2</i>               | Mesenchyme homeobox 2  | 2.34  | 9.0x10 <sup>3</sup> | 1.37        | 3.34                 | 4.58                   |
| <i>BATF3</i>               | Basic leucine zipper transcription factor, ATF-like 3  | 2.53  | 1.9x10 <sup>2</sup> | 1.20        | 5.86                 | 7.06                   |
| <i>KRT34</i>               | Keratin 34   | 2.89  | 2.0x10 <sup>4</sup> | 1.36        | 3.86                 | 5.25                   |
| <i>ST6GALNAC5</i>          | ST6 ( $\alpha$ -N-acetyl-neuraminy1-2,3- $\beta$ -galactosyl-1,3)-N-acetylglactosaminide $\alpha$ -2,6-sialyltransferase 5 | 2.39  | 2.5x10 <sup>2</sup> | 1.40        | 3.93                 | 5.50                   |
| <i>SERPINB7</i>            | Serpin peptidase inhibitor, clade B (ovalbumin), member 7  | 2.05  | 5.9x10 <sup>2</sup> | 1.46        | 4.92                 | 7.18                   |
| <i>CRISPLD2</i>            | Cysteine-rich secretory protein LCCL domain containing 2   | 2.49  | 2.4x10 <sup>2</sup> | 1.22        | 5.83                 | 7.14                   |
| <i>LOC644656</i>           | Uncharacterized LOC644656  | 4.97  | 4.0x10 <sup>4</sup> | 1.22        | 5.94                 | 7.25                   |
| <i>FRMD6-AS1</i>           | FRMD6 antisense RNA 1  | 3.57  | 2.2x10 <sup>3</sup> | 1.21        | 5.11                 | 6.19                   |

Table VII. Continued.

| Gene                | Description   | Score | P-value              | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|---------------------|---|-------|----------------------|-------------|----------------------|------------------------|
| <i>MGLL</i>         | Monoglyceride lipase  | 3.50  | 2.6x10 <sup>3</sup>  | 1.27        | 7.44                 | 9.49                   |
| <i>CYP2R1</i>       | Cytochrome P450, family 2, subfamily R, polypeptide                                       | 2.47  | 2.5x10 <sup>2</sup>  | 1.30        | 6.37                 | 8.25                   |
| <i>C11orf41</i>     | 1 KIAA1549-like   | 2.16  | 4.2x10 <sup>2</sup>  | 1.21        | 4.64                 | 5.62                   |
| <i>LOC389906</i>    | Zinc finger protein 839 pseudogene  | 2.01  | 6.2x10 <sup>2</sup>  | 1.36        | 5.07                 | 6.89                   |
| <i>ATP8B1</i>       | ATPase, aminophospholipid transporter, class I, type 8B, member 1                         | 2.75  | 1.7x10 <sup>2</sup>  | 1.35        | 6.06                 | 8.21                   |
| <i>EXT1</i>         | Exostosin glycosyltransferase 1   | 3.64  | 1.4x10 <sup>3</sup>  | 1.20        | 9.00                 | 10.82                  |
| <i>APCDD1L</i>      | Adenomatosis polyposis coli downregulated 1-like  | 2.30  | 3.5x10 <sup>2</sup>  | 1.27        | 5.46                 | 6.92                   |
| <i>LOC100506325</i> | Uncharacterized LOC100506325  | 4.38  | 1.2x10 <sup>3</sup>  | 1.26        | 4.92                 | 6.20                   |
| <i>MCM3AP-AS1</i>   | MCM3AP antisense RNA 1  | 3.24  | 6.6x10 <sup>3</sup>  | 1.20        | 5.13                 | 6.14                   |
| <i>C10orf47</i>     | Proline and serine-rich protein 2   | 3.44  | 4.8x10 <sup>3</sup>  | 1.52        | 4.17                 | 6.34                   |
| <i>AFAP1L2</i>      | Actin filament associated protein 1-like 2  | 2.78  | 1.3x10 <sup>2</sup>  | 1.43        | 4.34                 | 6.21                   |
| <i>PARP8</i>        | Poly (ADP-ribose) polymerase family, member 8   | 2.52  | 2.3x10 <sup>2</sup>  | 1.22        | 5.07                 | 6.20                   |
| <i>UGT8</i>         | UDP glycosyltransferase 8   | 2.18  | 4.3x10 <sup>2</sup>  | 1.28        | 5.39                 | 6.91                   |
| <i>LOC730755</i>    | LOC730755   | 2.39  | 3.2x10 <sup>3</sup>  | 1.55        | 4.44                 | 6.87                   |
| <i>HBE1</i>         | Hemoglobin, epsilon 1   | 2.56  | 1.7x10 <sup>2</sup>  | 1.48        | 4.71                 | 6.98                   |
| <i>MPP4</i>         | Membrane protein, palmitoylated 4 (MAGUK p55 subfamily member 4)                          | 2.89  | 1.6x10 <sup>3</sup>  | 1.42        | 3.50                 | 4.96                   |
| <i>CSTA</i>         | Cystatin A (stefin A)   | 2.04  | 4.5x10 <sup>2</sup>  | 1.39        | 3.80                 | 5.29                   |
| <i>SRGN</i>         | Serglycin   | 2.40  | 2.7x10 <sup>2</sup>  | 1.45        | 7.05                 | 10.24                  |
| <i>LOC100506465</i> | Uncharacterized LOC100506465  | 2.55  | 1.7x10 <sup>2</sup>  | 1.30        | 4.40                 | 5.72                   |
| <i>MOK</i>          | MOK protein kinase  | 2.00  | 5.8x10 <sup>2</sup>  | 1.21        | 6.81                 | 8.25                   |
| <i>INPP4B</i>       | Inositol polyphosphate-4-phosphatase, type II, 105 kDa                                    | 2.59  | 2.0x10 <sup>2</sup>  | 1.37        | 5.53                 | 7.60                   |
| <i>AFAP1L1</i>      | Actin filament associated protein 1-like 1  | 2.21  | 3.9x10 <sup>2</sup>  | 1.23        | 4.77                 | 5.87                   |
| <i>CCBE1</i>        | Collagen and calcium binding EGF domains 1  | 2.07  | 5.5x10 <sup>2</sup>  | 1.37        | 4.55                 | 6.25                   |
| <i>KCNK1</i>        | Potassium channel, subfamily K, member 1  | 1.49  | 2.2x10 <sup>1</sup>  | 1.22        | 3.76                 | 4.61                   |
| <i>CCND2</i>        | Cyclin D2   | 2.31  | 1.5x10 <sup>2</sup>  | 1.35        | 3.98                 | 5.36                   |
| <i>CDA</i>          | Cytidine deaminase  | 1.43  | 1.6x10 <sup>1</sup>  | 1.04        | 7.58                 | 7.86                   |
| <i>DMKN</i>         | Dermokine   | 2.03  | 5.7x10 <sup>2</sup>  | 1.36        | 4.52                 | 6.16                   |
| <i>NOG</i>          | Noggin  | 2.06  | 5.1x10 <sup>2</sup>  | 1.44        | 4.04                 | 5.82                   |
| <i>GTSF1</i>        | Gametocyte specific factor 1  | 2.02  | 6.6x10 <sup>2</sup>  | 1.59        | 4.07                 | 6.47                   |
| <i>NT5E</i>         | 5'-nucleotidase, ecto (CD73)  | 2.73  | 1.2x10 <sup>2</sup>  | 1.24        | 8.11                 | 10.04                  |
| <i>BIRC3</i>        | Baculoviral IAP repeat containing 3   | 2.07  | 5.4x10 <sup>2</sup>  | 1.24        | 4.88                 | 6.05                   |
| <i>NAP1L2</i>       | Nucleosome assembly protein 1-like 2  | 2.47  | 2.3x10 <sup>2</sup>  | 1.31        | 4.87                 | 6.36                   |
| <i>SLCO4A1</i>      | Solute carrier organic anion transporter family, member 4A1                               | 2.39  | 3.1x10 <sup>2</sup>  | 1.30        | 6.35                 | 8.26                   |
| <i>KIAA1324L</i>    | KIAA1324-like   | 2.14  | 4.9x10 <sup>2</sup>  | 1.19        | 4.75                 | 5.66                   |
| <i>CYP2J2</i>       | Cytochrome P450, family 2, subfamily J, polypeptide 2                                     | 3.00  | 8.8x10 <sup>3</sup>  | 1.28        | 4.13                 | 5.27                   |
| <i>TUBA3C</i>       | Tubulin, $\alpha$ 3c  | 2.44  | 2.7x10 <sup>2</sup>  | 1.20        | 5.59                 | 6.70                   |
| <i>CTAG2</i>        | Cancer/testis antigen 2   | 2.08  | 7.2x10 <sup>2</sup>  | 1.35        | 3.85                 | 5.21                   |
| <i>GALNTL4</i>      | UDP-N-acetyl- $\alpha$ -D-galactosamine: polypeptide-N-acetylgalactosaminyltransferase 18 | 2.52  | 2.2x10 <sup>02</sup> | 1.26        | 5.66                 | 7.10                   |
| <i>MGC16121</i>     | MIR503 host gene (non-protein coding)   | 2.81  | 1.2x10 <sup>2</sup>  | 1.25        | 5.74                 | 7.18                   |
| <i>COL3A1</i>       | Collagen, type III, $\alpha$ 1  | 2.32  | 3.3x10 <sup>2</sup>  | 1.53        | 5.05                 | 7.74                   |
| <i>PAPSS2</i>       | 3'-phosphoadenosine 5'-phosphosulfate synthase 2  | 1.98  | 6.9x10 <sup>2</sup>  | 1.25        | 7.17                 | 8.98                   |
| <i>BDNF-AS1</i>     | BDNF antisense RNA  | 2.91  | 9.6x10 <sup>3</sup>  | 1.25        | 4.12                 | 5.16                   |
| <i>KRTAP1-5</i>     | Keratin associated protein 1-5  | 2.40  | 2.6x10 <sup>3</sup>  | 1.37        | 4.06                 | 5.55                   |
| <i>CCDC80</i>       | Coiled-coil domain containing 80  | 2.54  | 2.2x10 <sup>2</sup>  | 1.29        | 6.78                 | 8.73                   |
| <i>NAP1L3</i>       | Nucleosome assembly protein 1-like 3  | 2.06  | 5.6x10 <sup>2</sup>  | 1.29        | 5.73                 | 7.39                   |

Table VII. Continued.

| Gene             | Description                                | Score | P-value             | Fold-change | Up <sup>a</sup> mean | Down <sup>a</sup> mean |
|------------------|--|-------|---------------------|-------------|----------------------|------------------------|
| <i>TMEM171</i>   | Transmembrane protein 171                  | 2.88  | 1.1x10 <sup>2</sup> | 1.40        | 4.45                 | 6.22                   |
| <i>NAV3</i>      | Neuron navigator 3                         | 2.59  | 1.5x10 <sup>2</sup> | 1.33        | 5.05                 | 6.70                   |
| <i>HIST1H4H</i>  | Histone cluster 1, H4h                     | 2.50  | 1.5x10 <sup>2</sup> | 1.21        | 4.38                 | 5.30                   |
| <i>FCRLB</i>     | Fc receptor-like B                         | 2.89  | 1.3x10 <sup>2</sup> | 1.23        | 5.38                 | 6.60                   |
| <i>CSPG4</i>     | Chondroitin sulfate proteoglycan 4         | 2.54  | 2.3x10 <sup>2</sup> | 1.43        | 4.35                 | 6.22                   |
| <i>LINC00341</i> | Long intergenic non-protein coding RNA 341 | 1.97  | 7.1x10 <sup>2</sup> | 1.23        | 6.29                 | 7.75                   |
| <i>GAD1</i>      | Glutamate decarboxylase 1 (brain, 67 kDa)  | 2.12  | 5.5x10 <sup>2</sup> | 1.21        | 5.24                 | 6.34                   |

<sup>a</sup>Up, and down mean refers to the mean of the specific gene expression levels in the ten most PGC1 $\alpha$  up- or downregulated cell lines. MHC, Major histocompatibility complex.

Table VIII. Annotated summary of class B of peroxisome proliferator-activated receptor  $\gamma$ , coactivator 1 $\alpha$ .

| Functional role                                    | Genes | P-value              | -Log (P-value) |
|--|-------|----------------------|----------------|
| Biological process                                 |       |                      |                |
| MHCII-mediated immunity                            | 3     | 8.20x10 <sup>3</sup> | 2.09           |
| Signal transduction                                | 27    | 9.70x10 <sup>3</sup> | 2.01           |
| Intracellular signaling cascade                    | 11    | 1.10x10 <sup>2</sup> | 1.96           |
| Cell surface receptor mediated signal transduction | 16    | 1.40x10 <sup>2</sup> | 1.85           |
| T-cell mediated immunity                           | 5     | 1.40x10 <sup>2</sup> | 1.85           |
| Ligand-mediated signaling                          | 7     | 1.60x10 <sup>2</sup> | 1.80           |
| Calcium mediated signaling                         | 4     | 2.00x10 <sup>2</sup> | 1.70           |
| Other oncogenesis                                  | 3     | 4.30x10 <sup>2</sup> | 1.37           |
| Cell communication                                 | 11    | 6.90x10 <sup>2</sup> | 1.16           |
| Cellular component                                 |       |                      |                |
| MHC protein complex                                | 4     | 2.90x10 <sup>3</sup> | 2.54           |
| Extracellular matrix                               | 7     | 7.80x10 <sup>3</sup> | 2.11           |
| Extracellular region part                          | 12    | 8.40x10 <sup>3</sup> | 2.08           |
| MHC class II protein complex                       | 3     | 9.30x10 <sup>3</sup> | 2.03           |
| Extracellular region                               | 18    | 2.10x10 <sup>2</sup> | 1.68           |
| Proteinaceous extracellular matrix                 | 6     | 2.30x10 <sup>2</sup> | 1.64           |
| Apical plasma membrane                             | 4     | 2.90x10 <sup>2</sup> | 1.54           |
| Chromatin assembly complex                         | 2     | 3.00x10 <sup>2</sup> | 1.52           |
| Microsome  | 5     | 3.20x10 <sup>2</sup> | 1.49           |
| Vesicular fraction                                 | 5     | 3.50x10 <sup>2</sup> | 1.46           |
| Apical part of cell                                | 4     | 6.10x10 <sup>2</sup> | 1.21           |

The dataset of significantly changed genes were identified using the Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov>) (P<0.05). MHC, Major Histocompatibility Complex.

and motility. Class B contained genes associated with immunity, oncogenesis and signaling, including intracellular, T cell-mediated, ligand-mediated and-calcium mediated pathways. Class A genes are involved in mitochondrial and metabolic pathways, whilst class B genes are involved in differentiation and immune pathways. These data reinforce the hypothesis that *PGC1 $\alpha$*  may have an important role in regulating mitochondrial and metabolic signaling pathways in the GBM microenvironment.

A notable result was the association of *NDN* with *PGC1 $\alpha$* . *NDN* is reported to function as a tumor suppressor in GBM (29) and controls the proliferation of white adipose progenitor cells (30). *NDN* interacts with *PGC1 $\alpha$*  via nicotinamide adenine dinucleotide dependent protein deacetylase (Sirt-1) and two transcription factors, E2F1 and P53, suggesting that interactions with these cell cycle regulating factors are key to its function (31). Therefore, it is hypothesized that *PGC1 $\alpha$*



Table IX. Differentially regulated signaling pathways in classes A and B.

| Signaling pathways   | Number <sup>a</sup> | P-value              |
|--|---------------------|----------------------|
| Class A  |                     |                      |
| Electron transport reaction in mitochondria                            | 3                   | 2.1x10 <sup>-2</sup> |
| Shuttle for transfer of acetyl groups from mitochondria to the cytosol | 3                   | 2.8x10 <sup>-2</sup> |
| Role of PPAR-γ coactivators in obesity and thermogenesis               | 3                   | 3.5x10 <sup>-2</sup> |
| Class B  |                     |                      |
| Th1/Th2 differentiation  | 5                   | 6.3x10 <sup>-3</sup> |
| Cytokines and inflammatory response                                    | 5                   | 1.6x10 <sup>-2</sup> |
| Bystander B-cell activation  | 3                   | 3.6x10 <sup>-2</sup> |
| IL12- and Stat4-dependent signaling pathway in Th1 development         | 4                   | 4.0x10 <sup>-2</sup> |
| Dendritic cells in regulating Th1 and Th2 development                  | 4                   | 4.5x10 <sup>-2</sup> |

Using the Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov>) differentially regulated signaling pathways in class A and B were identified using the dataset of significantly changed genes (P<0.05). <sup>a</sup>Number of significantly changed genes per pathway. PPAR, peroxisome proliferator activated receptor; IL, interleukin 12; NF-κB, nuclear factor-κB; NK, natural killer; Th, T helper.

enhances antioxidant capacity in GBM by interacting with NDN and Sirt1, leading to delayed progression of necrosis and ultimately increasing overall patient survival. Future studies that elucidate the molecular interactions of PGC1α are required to derive improved insights into the diagnosis, prognosis and treatment of GBM.

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