Expression of PGC1α in glioblastoma multiforme patients

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Abstract. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) 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 PGC1α 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 PGC1α in GBM. It was observed that PGC1α 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 *PGC1α* mRNA expression. Notably, mitochondrial function-associated genes were highly expressed in cells alongside high *PGC1α* expression. Collectively, the results of the present study indicate that PGC1α is associated with mitochondrial dysfunction in GBM and may have a role in tumor pathogenesis and progression.

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Introduction

Peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) regulates metabolism (1,2), mitochondrial biogenesis and energy homeostasis (3,4). A number of studies have reported PGC1α 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, PGC1α in the central nervous system is less associated with energy state or thermogenesis (6). PGC1α expression in the central nervous system is high in the embryonic and early postnatal stages, but is decreased during maturation. PGC1α is expressed mostly by γ-aminobutyric acid-ergic neurons; however, a low level of PGC1α is also expressed in glia in the mature brain (7). There is a significant association between PGC1α and the metabolism of reactive oxygen species. PGC1α-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 PGC1α has a role in cellular antioxidant defense (8).

Numerous clinical studies have reported a significant association between PGC1α and a number of types of cancer. In breast, colon and ovarian cancer (9-12), a significant decrease in PGC1α 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 PGC1α expression is present in melanoma, with a corresponding decrease in patient survival (14). The role of PGC1α 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

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 PGC1α in normal cortical tissues and GBM tissues was compared. The results of the present study indicate that PGC1α 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-PGC1α 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 PGC1α (1:200; Santa Cruz Biotechnology, Inc.; #sc-13067). For immunofluorescence analysis, PGC1α 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 *PGC1α* 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 *PGC1α* (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 *PGC1α* 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) 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^2) 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 ± 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

PGC1α is highly and variably expressed in GBM patients. To determine the association between PGC1α and GBM, levels of PGC1α protein in GBM and control (normal cortex) tissues were compared using publicly available TMAs from US Biomax, Inc. (Fig. 1). PGC1α was weakly detectable in the nuclei of cortical tissues in the control, whereas it was highly and sporadically expressed throughout the GBM tissues. Furthermore, PGC1α was mostly expressed within the cytoplasm with pale nucleic density (Fig. 1A). Bright-field immunohistochemical analysis of TMA images using a densitometer revealed that PGC1α expression varied between tumor samples (Fig. 1B).

For additional validation, *PGC1α* mRNA levels were determined in GBM cell lines (n=38) using the Broad-Novartis CCLE database (21). Comparative analysis of *PGC1α*

Table I. Patient demographics and tumor characteristics.

Case no.	Age[a] (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

[a]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_2) to 8.83 (log_2), 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	PGC1α 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; PGC1α, proliferator-activated receptor γ coactivator 1α.

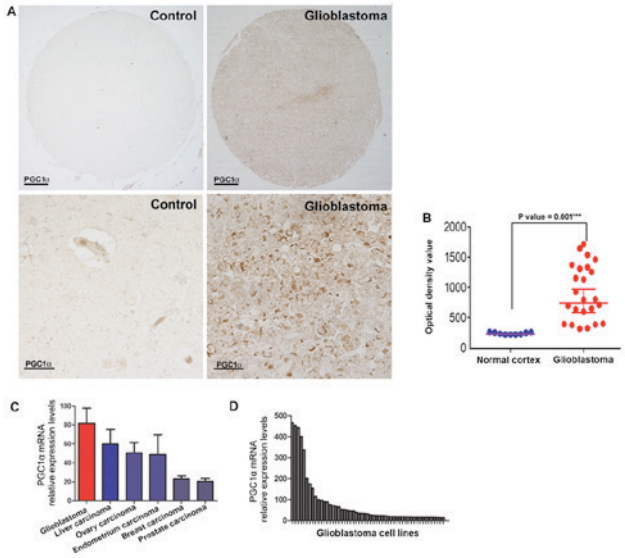

Figure 1. Expression level of PGC1α in glioblastoma multiforme and normal cortex. (A) Representative immunohistochemical analysis of PGC1α expression from the US Biomax, Inc. TMA database. Scale bar: 100 μm (upper panels) and 20 μm (lower panels). (B) Corrected optical density values of PGC1α in normal cortex tissues and glioblastoma tissues from the US Biomax, Inc. TMA database (unpaired t-test, ± SEM; ***P<0.001). (C) Relative mRNA expression of *PGC1α* across various cancer types in the CCLE database. Error bars: mean ± 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 *PGC1α* mRNA among 38 glioblastoma multiforme cell lines in the CCLE database. CCLE, cancer cell line encyclopedia; PGC1α, peroxisome proliferator-activated receptor γ, coactivator 1α; TMA, tissue microarrays; SEM, standard error of the mean.

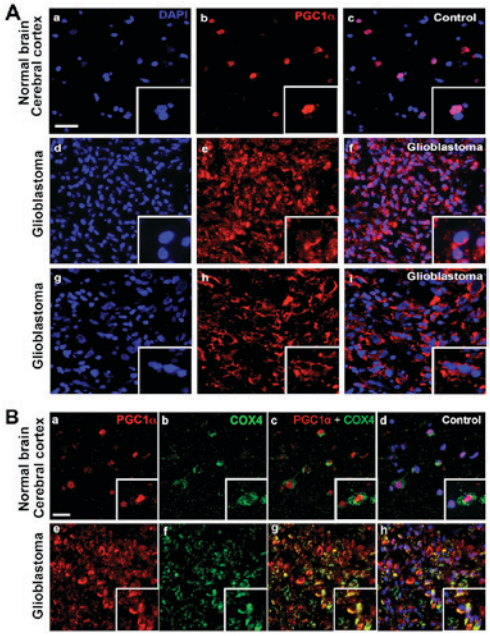

Figure 2. Colocalization of PGC1α with COX4 in GBM. (A) PGC1α localization in glioblastoma multiforme and normal cortex was analyzed using tissue microarrays. PGC1α expression in the normal cortex was localized to the nucleus, but perinuclear and cytoplasmic expression of PGC1α was observed in GBM. (B) Representative co-immunofluorescence staining of PGC1α (red) and COX4 (green) with counter-staining with DAPI (blue) in the normal cortex and GBM. PGC1α-positive cells were primarily co-labeled with the mitochondrial marker COX4 in GBM. COX4, cytochrome *c* oxidase subunit 4; GBM, glioblastoma multiforme; PGC1α, peroxisome proliferator-activated receptor γ.

with the finding that PGC1α is localized in the mitochondria in GBM as previously described.

PGC1α 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 *PGC1α* expression. The expression of TCA cycle-(P<0.0001),

Table III. List of Gene Neighbors of peroxisome proliferator-activated receptor γ coactivator 1α differentially expressed in glioblastoma multiforme cells.

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

Table III. Continued.

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

Table III. Continued.

Gene symbol	Description
Monosaccharide metabolic process	
PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
GYG2	Glycogenin 2
MDH1	Malate dehydrogenase 1, NAD (soluble)
OGDHL	Oxoglutarate dehydrogenase-like
PDHA1	Pyruvate dehydrogenase (lipoamide) α 1
Oxidoreduction coenzyme metabolic process	
COQ9	Coenzyme Q9 homolog (S. cerevisiae)
IDH3A	Isocitrate dehydrogenase 3 (NAD^+) α
MDH1	Malate dehydrogenase 1, NAD (soluble)
Unknown biological process	
CEND1	Cell cycle exit and neuronal differentiation 1
COX7B	Cytochrome c oxidase subunit VIIb
TMCC2	Transmembrane and coiled-coil domain family 2
SOX13	SRY (sex determining region Y)-box 13
BTBD3	BTB (POZ) domain containing 3
ZNF222	Zinc finger protein 222
DCUN1D2	DCN1, defective in cullin neddylation 1, domain containing 2
MFSD2A	Major facilitator superfamily domain containing 2A
CX3CL1	Chemokine (C-X3-C motif) ligand 1
GSTM4	Glutathione S-transferase mu 4
PIGA	Phosphatidylinositol glycan anchor biosynthesis, class A
ITPKB	Inositol-trisphosphate 3-kinase B
TSPAN16	Tetraspanin 16
CHCHD3	Coiled-coil-helix-coiled-coil-helix domain containing 3
APOO	Apolipoprotein O
AKAP11	A kinase (PRKA) anchor protein 11
NEBL	Nebulette
SCUBE3	Signal peptide, CUB domain, EGF-like 3
RRAGD	Ras-related GTP binding D
IGHV1-2	Immunoglobulin heavy variable 1-2
RRAGD	Ras-related GTP binding D
TRIM2	Tripartite motif containing 2
TLE6	Transducin-like enhancer of split 6 (E(sp1) homolog, *Drosophila*)
LINC00461	Long intergenic non-protein coding RNA 461
SLC25A25	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25
SLC25A11	Solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11
IVNS1ABP	Influenza virus NS1A binding protein
HEY1	Hairy/enhancer-of-split related with YRPW motif 1
NDRG2	NDRG family member 2
COX5B	Cytochrome c oxidase subunit Vb
MRPL34	Mitochondrial ribosomal protein L34
STK32A	Serine/threonine kinase 32A
MEGF8	Multiple EGF-like-domains 8
ATP1A1	ATPase, Na^+/K^+ transporting, α 1 polypeptide
RBPMS2	RNA binding protein with multiple splicing 2
LPL	Lipoprotein lipase
FURIN	Furin (paired basic amino acid cleaving enzyme)
ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1
KLHL15	Kelch-like family member 15
BTBD1	BTB (POZ) domain containing 1
PTCD3	Pentatricopeptide repeat domain 3

Table III. Continued.

Gene symbol	Description
RBM38	RNA binding motif protein 38
LYNX1	Ly6/neurotoxin 1
EFHA1	Mitochondrial calcium uptake 2
NCOA1	Nuclear receptor coactivator 1
KIF13B	Kinesin family member 13B
FAM199X	Family with sequence similarity 199, X-linked
RPRM	Reprimo, TP53 dependent G2 arrest mediator candidate
ZNF462	Zinc finger protein 462
ANXA13	Annexin A13
SPG20OS	SPG20 opposite strand
GPR98	G protein-coupled receptor 98
GK	Glycerol kinase
UCK1	Uridine-cytidine kinase 1
LNX2	Ligand of numb-protein X 2
SPG20	Spastic paraplegia 20 (Troyer syndrome)
WNK3	WNK lysine deficient protein kinase 3
LOC100506108	LOC100506108
GCNT2	Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)
SLC31A1	Solute carrier family 31 (copper transporter), member 1
OSTM1	Osteopetrosis associated transmembrane protein 1
TMF1	TATA element modulatory factor 1
TSPAN3	Tetraspanin 3
COL4A3	Collagen, type IV, α3 (Goodpasture antigen)
GPM6B	Glycoprotein M6B
PELI2	Pellino E3 ubiquitin protein ligase family member 2
LOC401431	LOC401431
UBAC1	UBA domain containing 1
ATG4D	Autophagy related 4D, cysteine peptidase
COMMD6	COMM domain containing 6
FAM65B	Family with sequence similarity 65, member B
TMEM2	Transmembrane protein 2
ASB9	Ankyrin repeat and SOCS box containing 9
BCAM	Basal cell adhesion molecule (Lutheran blood group)
KIF16B	Kinesin family member 16B
CHKA	Choline kinase α
PPM1E	Protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1E
CA2	Carbonic anhydrase II

oxidative phosphorylation (OXPHOS)-(P<0.0001) and lipogenesis-associated genes (P<0.01) was significantly increased in the *PGC1α*-upregulated cells compared with the *PGC1α*-downregulated cells (Fig. 4A-C). Furthermore, the expression of antioxidant-associated genes was significantly increased in the *PGC1α*-upregulated cell lines compared with the *PGC1α*-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 *PGC1α*. Notably, genes associated with mitochondrial functions, including TCA cycle, OXPHOS, lipogenesis and antioxidant genes, were highly expressed in cells with high *PGC1α* 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 PGC1α up- and downregulated GBM cell lines. Bioinformatics analysis using Class Neighbors yielded two classes of GBM cell lines. Class A contained the ten most *PGC1α*-upregulated GBM cell lines, and class B contained the ten most *PGC1α*-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 γ coactivator 1α.

Functional role	Genes	P-value	-Log (P-value)
Biological process			
Generation of precursor metabolites and energy	12	$5.50x10^{7}$	6.26
Oxidation reduction	13	$9.60x10^{5}$	4.02
Energy derivation by oxidation of organic compounds	7	$9.80x10^{5}$	4.01
Cellular respiration	6	$1.40x10^{4}$	3.85
Acetyl-CoA metabolic process	4	$5.40x10^{4}$	3.27
Coenzyme metabolic process	6	$1.20x10^{3}$	2.92
Oxidative phosphorylation	5	$1.60x10^{3}$	2.80
Cofactor metabolic process	6	$3.40x10^{3}$	2.47
Tricarboxylic acid cycle	3	$6.20x10^{3}$	2.21
Acetyl-CoA catabolic process	3	$6.20x10^{3}$	2.21
Coenzyme catabolic process	3	$7.90x10^{3}$	2.10
Cofactor catabolic process	3	$1.10x10^{2}$	1.96
Aerobic respiration	3	$1.40x10^{2}$	1.85
Hexose metabolic process	5	$1.70x10^{2}$	1.77
Mitochondrial electron transport, NADH to ubiquinone	3	$2.00x10^{2}$	1.70
Glycolysis	3	$2.50x10^{2}$	1.60
Monosaccharide metabolic process	5	$2.80x10^{2}$	1.55
Oxidoreduction coenzyme metabolic process	3	$3.00x10^{2}$	1.52
Cellular component			
Mitochondrial part	22	$3.40x10^{12}$	11.47
Mitochondrion	25	$1.20x10^{9}$	8.92
Mitochondrial envelope	16	$5.90x10^{9}$	8.23
Mitochondrial inner membrane	14	$9.20x10^{9}$	8.04
Mitochondrial membrane	15	$2.20x10^{8}$	7.66
Organelle inner membrane	14	$2.20x10^{8}$	7.66
Organelle envelope	16	$9.80x10^{7}$	6.01
Envelope	16	$1.00x10^{6}$	6.00
Mitochondrial lumen	9	$3.20x10^{5}$	4.49
Mitochondrial matrix	9	$3.20x10^{5}$	4.49
Organelle membrane	18	$6.40x10^{5}$	4.19
Mitochondrial membrane part	6	$6.00x10^{4}$	3.22
Mitochondrial respiratory chain	4	$5.20x10^{3}$	2.28
Respiratory chain	4	$8.00x10^{3}$	2.10
Respiratory chain complex I	3	$2.20x10^{2}$	1.66
Mitochondrial respiratory chain complex I	3	$2.20x10^{2}$	1.66
NADH dehydrogenase complex	3	$2.20x10^{2}$	1.66
Cell surface	6	$4.20x10^{2}$	1.38
Mitochondrial proton-transporting ATP synthase complex	2	$9.90x10^{2}$	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 *PGC1α*, 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 PGC1α and GBM, and

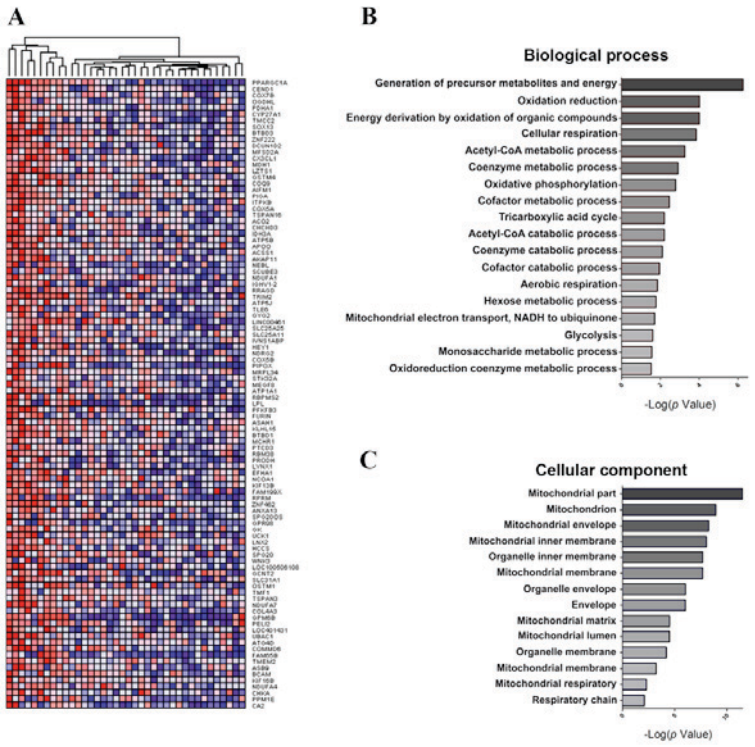

Figure 3. Bioinformatics analysis of *PGC1α*-associated genes in GBM cells lines. (A) Hierarchical clustering of *PGC1α* Gene Neighbors in GBM cell lines. GBM cells are arranged in decreasing order of *PGC1α* mRNA expression by Pearson distance. Colors in the heat-map represent expression relative to the mean expression value, with red indicating higher expression and blue indicating lower expression. Gene neighbors of *PGC1α* are displayed in the right column. Gene Neighbors of *PGC1α* were characterized as either (B) biological processes or (C) cellular components by gene ontology enrichment analysis. GBM, glioblastoma multiforme; PGC1α, peroxisome proliferator-activated receptor γ, coactivator 1α.

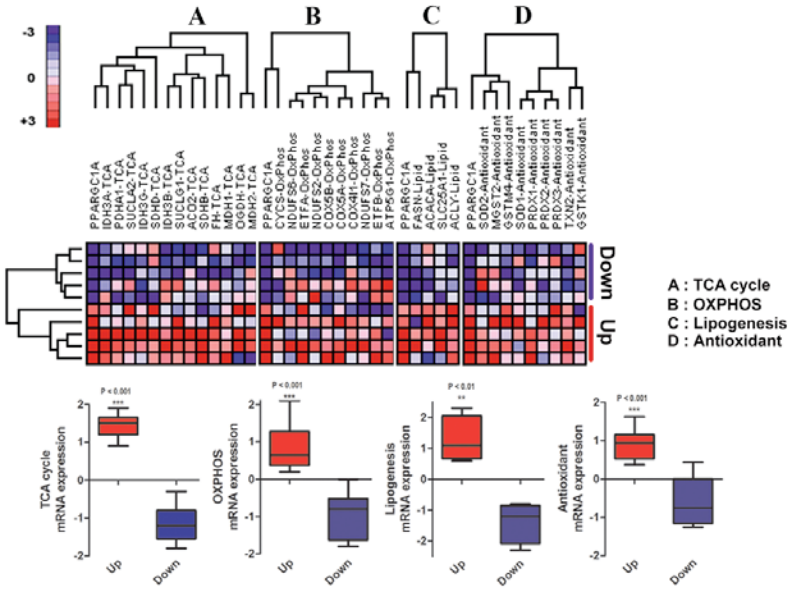

Figure 4. Two-way hierarchical clustering of target gene sets previously reported to be associated with *PGC1α* in cancer. (A) *TCA* cycle genes (***P<0.001), (B) *OXPHOS* genes (***P<0.001), (C) Lipogenesis genes (**P<0.01) and (D) Antioxidant genes (***P<0.001) are differentially expressed among the top five *PGC1α* up- and downregulated GBM cell lines. The top five GBM cell lines are LNZ308, 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; PGC1α, peroxisome proliferator-activated receptor γ, coactivator 1α; TCA, tricarboxylic acid.

the role PGC1α may have in patient survival. Protein level data demonstrated that PGC1α expression was increased in a subpopulation of tumor cells, although there were variations between different GBM cell lines and patients. PGC1α 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 γ coactivator 1α-upregulated glioblastoma multiforme cells.

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

Table V. Continued.

Gene	Description	Score	P-value	Fold-change	Up[a] mean	Down[a] mean
PDE4B	Phosphodiesterase 4B, cAMP-specific	2.59	$2.1x10^{2}$	1.26	6.83	5.42
PDGFRL	Platelet-derived growth factor receptor-like	2.55	$2.5x10^{2}$	1.29	6.76	5.22
SFRP1	Secreted frizzled-related protein 1	2.33	$3.5x10^{2}$	1.42	7.77	5.46
SCG2	Secretogranin II	2.50	$2.0x10^{2}$	1.43	8.08	5.64
SCUBE3	Signal peptide, CUB domain, EGF-like 3	2.63	$3.6x10^{3}$	1.34	7.76	5.78
TLR4	Toll-like receptor 4	2.82	$9.8x10^{3}$	1.36	6.29	4.61
TMTC1	Transmembrane and tetratricopeptide repeat containing 1	2.43	$2.5x10^{2}$	1.29	6.10	4.72
Ectoderm development						
EPHA3	EPH receptor A3	2.45	$1.9x10^{2}$	1.39	5.33	3.83
CDH6	Cadherin 6, type 2, K-cadherin (fetal kidney)	2.80	$1.1x10^{2}$	1.35	5.83	4.31
CELSR2	Cadherin, EGF LAG seven-pass G-type receptor 2	2.80	$1.2x10^{2}$	1.21	7.78	6.42
CXCR4	Chemokine (C-X-C motif) receptor 4	2.55	$1.5x10^{2}$	1.44	6.04	4.20
FABP7	Fatty acid binding protein 7, brain	2.26	$3.1x10^{2}$	1.57	6.87	4.38
FOXA2	Forkhead box A2	2.18	$6.2x10^{2}$	1.36	5.56	4.09
GPM6B	Glycoprotein M6B	2.14	$4.8x10^{2}$	1.46	7.60	5.19
HES1	Hairy and enhancer of split 1, (*Drosophila*)	3.29	$4.2x10^{3}$	1.21	8.42	6.98
HEY1	Hairy/enhancer-of-split related with YRPW motif 1	2.49	$2.3x10^{2}$	1.28	8.16	6.39
IRX1	Iroquois homeobox 1	2.81	$8.4x10^{3}$	1.48	6.61	4.47
JAG1	Jagged 1	3.16	$6.0x10^{3}$	1.22	7.89	6.48
NRG2	Neuregulin 2	2.73	$1.4x10^{2}$	1.22	4.99	4.09
NRP2	Neuropilin 2	2.75	$1.2x10^{2}$	1.25	6.74	5.40
Cell structure and motility						
CELSR2	Cadherin, EGF LAG seven-pass G-type receptor 2	2.80	$1.2x10^{2}$	1.21	7.78	6.42
CXCR4	Chemokine (C-X-C motif) receptor 4	2.55	$1.5x10^{2}$	1.44	6.04	4.20
COL7A1	Collagen, type VII, α 1	2.53	$2.0x10^{2}$	1.26	8.29	6.58
DCLK1	Doublecortin-like kinase 1	2.69	$1.6x10^{2}$	1.21	4.91	4.06
DNM3	Dynamin 3	2.22	$3.7x10^{2}$	1.21	6.13	5.06
DYNC1I1	Dynein, cytoplasmic 1, intermediate chain 1	2.85	$1.1x10^{2}$	1.42	7.89	5.55
FOXA2	Forkhead box A2	2.18	$6.2x10^{2}$	1.36	5.56	4.09
GPM6B	Glycoprotein M6B	2.14	$4.8x10^{2}$	1.46	7.60	5.19
ITPR1	Inositol 1,4,5-trisphosphate receptor, type 1	2.68	$1.4x10^{2}$	1.25	6.99	5.60
JAG1	Jagged 1	3.16	$6.0x10^{3}$	1.22	7.89	6.48
MYL5	Myosin, light chain 5, regulatory	3.19	$5.6x10^{3}$	1.25	6.73	5.40
PRICKLE2	Prickle homolog 2 (*Drosophila*)	2.46	$2.3x10^{2}$	1.22	8.15	6.70
SPP1	Secreted phosphoprotein 1	0.82	$4.2x10^{1}$	1.03	7.03	7.22
Neurogenesis						
EPHA3	EPH receptor A3	2.45	$1.9x10^{2}$	1.39	5.33	3.83
CDH6	Cadherin 6, type 2, K-cadherin (fetal kidney)	2.80	$1.1x10^{2}$	1.35	5.83	4.31
CELSR2	Cadherin, EGF LAG seven-pass G-type receptor 2	2.80	$1.2x10^{2}$	1.21	7.78	6.42
CXCR4	Chemokine (C-X-C motif) receptor 4	2.55	$1.5x10^{2}$	1.44	6.04	4.20
FOXA2	Forkhead box A2	2.18	$6.2x10^{2}$	1.36	5.56	4.09
GPM6B	Glycoprotein M6B	2.14	$4.8x10^{2}$	1.46	7.60	5.19
HES1	Hairy and enhancer of split 1, (*Drosophila*)	3.29	$4.2x10^{3}$	1.21	8.42	6.98
HEY1	Hairy/enhancer-of-split related with YRPW motif 1	2.49	$2.3x10^{2}$	1.28	8.16	6.39
IRX1	Iroquois homeobox 1	2.81	$8.4x10^{3}$	1.48	6.61	4.47
JAG1	Jagged 1	3.16	$6.0x10^{3}$	1.22	7.89	6.48
NRG2	Neuregulin 2	2.73	$1.4x10^{2}$	1.22	4.99	4.09
NRP2	Neuropilin 2	2.75	$1.2x10^{2}$	1.25	6.74	5.40

Table V. Continued.

Gene	Description	Score	P-value	Fold-change	Up[a] mean	Down[a] mean
Cell communication						
CDH6	Cadherin 6, type 2, K-cadherin (fetal kidney)	2.80	$1.1x10^{2}$	1.35	5.83	4.31
CELSR2	Cadherin, EGF LAG seven-pass G-type receptor 2	2.80	$1.2x10^{2}$	1.21	7.78	6.42
FABP7	Fatty acid binding protein 7, brain	2.26	$3.1x10^{2}$	1.57	6.87	4.38
FBLN1	Fibulin 1	2.62	$1.9x10^{2}$	1.27	7.35	5.78
FOXA2	Forkhead box A2	2.18	$6.2x10^{2}$	1.36	5.56	4.09
ITPR1	Inositol 1,4,5-trisphosphate receptor, type 1	2.68	$1.4x10^{2}$	1.25	6.99	5.60
NRG2	Neuregulin 2	2.73	$1.4x10^{2}$	1.22	4.99	4.09
SFRP1	Secreted frizzled-related protein 1	2.33	$3.5x10^{2}$	1.42	7.77	5.46
SCG2	Secretogranin II	2.50	$2.0x10^{2}$	1.43	8.08	5.64
SCUBE3	Signal peptide, CUB domain, EGF-like 3	2.63	$3.6x10^{3}$	1.34	7.76	5.78
TMTC1	Transmembrane and tetratricopeptide repeat containing 1	2.43	$2.5x10^{2}$	1.29	6.10	4.72
Mesoderm development						
EFHD1	EF-hand domain family, member D1	2.22	$4.4x10^{2}$	1.31	6.03	4.61
EPHA3	EPH receptor A3	2.45	$1.9x10^{2}$	1.39	5.33	3.83
FBLN1	Fibulin 1	2.62	$1.9x10^{2}$	1.27	7.35	5.78
FOXA2	Forkhead box A2	2.18	$6.2x10^{2}$	1.36	5.56	4.09
MYL5	Myosin, light chain 5, regulatory	3.19	$5.6x10^{3}$	1.25	6.73	5.40
NRP2	Neuropilin 2	2.75	$1.2x10^{2}$	1.25	6.74	5.40
PTHLH	Parathyroid hormone-like hormone	2.46	$1.9x10^{2}$	1.42	6.77	4.75
SCUBE3	Signal peptide, CUB domain, EGF-like 3	2.63	$3.6x10^{3}$	1.34	7.76	5.78
Cell structure						
CELSR2	Cadherin, EGF LAG seven-pass G-type receptor 2	2.80	$1.2x10^{2}$	1.21	7.78	6.42
COL7A1	Collagen, type VII, α1	2.53	$2.0x10^{2}$	1.26	8.29	6.58
DCLK1	Doublecortin-like kinase 1	2.69	$1.6x10^{2}$	1.21	4.91	4.06
DNM3	Dynamin 3	2.22	$3.7x10^{2}$	1.21	6.13	5.06
DYNC1I1	Dynein, cytoplasmic 1, intermediate chain 1	2.85	$1.1x10^{2}$	1.42	7.89	5.55
FOXA2	Forkhead box A2	2.18	$6.2x10^{2}$	1.36	5.56	4.09
GPM6B	Glycoprotein M6B	2.14	$4.8x10^{2}$	1.46	7.60	5.19
SPP1	Secreted phosphoprotein 1	0.82	$4.2x10^{1}$	1.03	7.03	7.22
Unknown biological process						
RNF182	Ring finger protein 182	2.22	$3.9x10^{2}$	1.27	8.41	6.64
ACSS3	Acyl-CoA synthetase short-chain family member 3	2.48	$3.3x10^{2}$	1.28	6.52	5.08
GSTM4	Glutathione S-transferase mu 4	4.79	$4.0x10^{4}$	1.41	7.93	5.62
LINC00461	Long intergenic non-protein coding RNA 461	4.67	$6.0x10^{4}$	1.55	9.31	5.99
FAM70A	Transmembrane protein 255A	3.80	$6.0x10^{4}$	1.72	7.46	4.33
COL21A1	Collagen, type XXI, α1	4.49	$4.0x10^{4}$	1.74	7.61	4.38
METTL7A	Methyltransferase like 7A	3.32	$5.0x10^{3}$	1.49	8.06	5.40
GMPR	Guanosine monophosphate reductase	0.33	$7.5x10^{1}$	1.01	8.81	8.94
NID1	Nidogen 1	2.36	$2.8x10^{2}$	1.26	9.12	7.23
KIAA0895	KIAA0895	2.04	$5.5x10^{2}$	1.21	6.57	5.44
C8orf4	Chromosome 8 open reading frame 4	0.91	$3.7x10^{1}$	1.04	10.02	9.67
SEL1L3	Sel-1 suppressor of lin-12-like 3 (*Caenorhabditis elegans*)	2.19	$4.3x10^{2}$	1.33	8.99	6.76
GPC4	Glypican 4	2.55	$2.2x10^{2}$	1.41	8.55	6.07
PLEKHG1	Pleckstrin homology domain containing, family G (with RhoGef domain) member 1	2.47	$2.8x10^{2}$	1.38	6.36	4.62

Table V. Continued.

Gene	Description	Score	P-value	Fold-change	Up[a] mean	Down[a] mean
PIPOX	Pipecolic acid oxidase	3.29	$4.0x10^4$	1.68	6.46	3.84
FAM65B	Family with sequence similarity 65, member B	2.56	$1.1x10^2$	1.39	5.57	3.99
C7orf57	Chromosome 7 open reading frame 57	2.17	$4.2x10^2$	1.46	5.56	3.80
PPP2R2B	Protein phosphatase 2, regulatory subunit B, β	3.58	$2.8x10^3$	1.61	7.44	4.62
SERP2	Stress-associated endoplasmic reticulum protein family member 2	2.11	$5.2x10^2$	1.22	6.19	5.09
SOX2	SRY (sex determining region Y)-box 2	1.23	$2.5x10^1$	1.04	4.07	3.92
RPRM	Reprimo, TP53 dependent G2 arrest mediator candidate	0.43	$6.9x10^1$	1.01	3.99	4.04
MFSD2A	Major facilitator superfamily domain containing 2A	3.69	$2.0x10^3$	1.30	7.33	5.63
PELI2	Pellino E3 ubiquitin protein ligase family member 2	2.91	$1.1x10^2$	1.29	7.33	5.68
GCNT2	Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)	2.40	$3.3x10^2$	1.22	7.59	6.22
SLC16A4	Solute carrier family 16, member 4	2.88	$1.1x10^2$	1.39	8.00	5.77
SH3BGR	SH3 domain binding glutamic acid-rich protein	1.58	$1.3x10^1$	1.05	10.64	10.12
WDR31	WD repeat domain 31	3.54	$2.8x10^3$	1.20	5.83	4.86
SLC16A9	Solute carrier family 16, member 9	2.07	$4.4x10^2$	1.23	6.40	5.19
GSTT1	Glutathione S-transferase theta 1	2.91	$1.3x10^2$	1.40	7.41	5.31
NDP	Norrie disease (pseudoglioma)	2.53	$2.4x10^2$	1.50	7.62	5.09
NDN	Necdin, melanoma antigen (MAGE) family member	2.42	$2.9x10^2$	1.44	7.59	5.27
ASB9	Ankyrin repeat and SOCS box containing 9	2.20	$4.3x10^2$	1.26	7.03	5.58
LONRF2	LON peptidase N-terminal domain and ring finger 2	2.08	$6.0x10^2$	1.37	6.10	4.44
SPHAR	S-phase response (cyclin related)	2.62	$1.8x10^2$	1.22	7.49	6.12
RNF144A	Ring finger protein 144A	2.62	$1.6x10^2$	1.24	7.07	5.71
SERINC5	Serine incorporator 5	4.07	$1.4x10^3$	1.20	10.73	8.95
RRAGD	Ras-related GTP binding D	2.42	$3.0x10^2$	1.28	8.29	6.48
OGDHL	Oxoglutarate dehydrogenase-like	2.65	$1.5x10^2$	1.25	6.36	5.11
CEND1	Cell cycle exit and neuronal differentiation 1	3.91	$1.0x10^3$	1.24	6.38	5.14
RBPMS2	RNA binding protein with multiple splicing 2	2.11	$4.6x10^2$	1.26	6.34	5.03
SULF2	Sulfatase 2	2.69	$1.9x10^2$	1.50	8.01	5.33
MMP7	Matrix metallopeptidase 7 (matrilysin, uterine)	2.97	$2.0x10^3$	1.24	5.14	4.15
SLC2A12	Solute carrier family 2 (facilitated glucose transporter), member 12	2.95	$8.4x10^3$	1.35	6.31	4.67
GFPT2	Glutamine-fructose-6-phosphate transaminase 2	2.24	$3.7x10^2$	1.29	8.35	6.46
SOX9	SRY (sex determining region Y)-box 9	2.18	$4.3x10^2$	1.31	9.42	7.17
C5orf46	Chromosome 5 open reading frame 46	2.29	$3.2x10^2$	1.34	8.92	6.67
CP	Ceruloplasmin (ferroxidase)	2.35	$3.3x10^2$	1.05	4.24	4.03
GPNMB	Glycoprotein (transmembrane) nmb	2.85	$1.1x10^2$	1.35	10.04	7.46
SERPINI1	Serpin peptidase inhibitor, clade I (neuroserpin), member 1	2.35	$3.5x10^2$	1.32	7.42	5.63
TPRG1	Tumor protein p63 regulated 1	2.36	$3.5x10^2$	1.30	5.12	3.94
PITX2	Paired-like homeodomain 2	2.09	$5.6x10^2$	1.32	5.44	4.13

[a]Up, and down mean refers to the mean of the specific gene expression levels in the ten most PGC1a up- or downregulated cell lines.

with a previous study that detected a brain-specific isoform of PGC1α in the cytoplasm rather than the nucleus (27). It was also reported that the PGC1α 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 PGC1α was expressed in the mitochondria of GBM cells. Based on these

Table VI. Annotated summary of class A of peroxisome proliferator-activated receptor γ coactivator 1α.

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

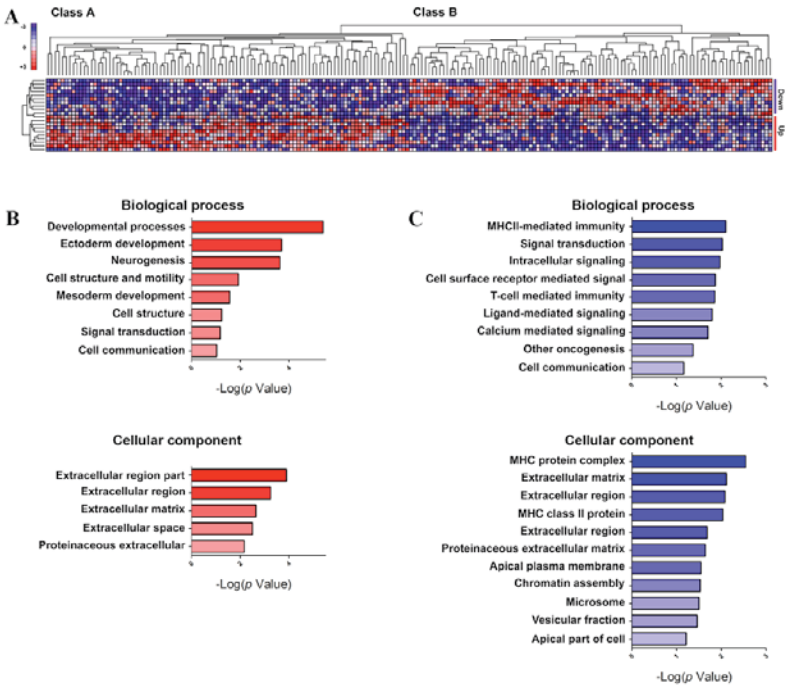

Figure 5. Bioinformatics analysis of *PGC1α*-associated genes in two classes of GBM cell lines. (A) Two-way hierarchical clustering of differentially expressed genes in the top ten *PGC1α* up- and downregulated GBM cell lines by Pearson distance. (B) Class A genes were divided into biological processes, 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' PGC1α, peroxisome proliferator-activated receptor γ, coactivator 1α.

corroborating results, it is predicted that PGC1α-mediated mitochondrial biogenesis and respiration is increased in GBM cells.

To investigate the role PGC1α has in GBM cells, several bioinformatics analyses were performed. The analyses demonstrated that metabolic and mitochondrial genes were highly correlated with *PGC1α* in a number of GBM cell lines. Class Neighbors analysis classified *PGC1α*-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 γ coactivator 1α downregulated glioblastoma multiforme cells.

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

Table VII. Continued.

Gene	Description	Score	P-value	Fold-change	Up[a] mean	Down[a] mean
IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte Maturation factor 1, p35)	3.58	$1.8x10^{3}$	1.26	4.24	5.35
IL4R	Interleukin 4 receptor	2.50	$1.7x10^{2}$	1.21	5.42	6.54
OXTR	Oxytocin receptor	2.29	$2.8x10^{2}$	1.31	5.90	7.70
PLCB4	Phospholipase C, β 4	2.66	$1.9x10^{2}$	1.31	6.48	8.50
PDGFA	Platelet-derived growth factor α polypeptide	2.29	$3.6x10^{2}$	1.26	6.68	8.43
Cell surface receptor mediated signal transduction						
ARAP2	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2	2.16	$4.9x10^{2}$	1.27	4.51	5.74
CD33	CD33 molecule	2.54	$6.6x10^{3}$	1.24	4.54	5.64
SLITRK5	SLIT and NTRK-like family, member 5	2.59	$1.6x10^{2}$	1.29	5.21	6.70
ADRB2	Adrenoceptor β 2, surface	3.28	$4.2x10^{3}$	1.34	5.85	7.85
F2RL2	Coagulation factor II (thrombin) receptor-like 2	2.24	$3.4x10^{2}$	1.39	4.33	6.04
FGF1	Fibroblast growth factor 1 (acidic)	2.06	$5.0x10^{2}$	1.31	4.35	5.69
GRB14	Growth factor receptor-bound protein 14	2.08	$4.8x10^{2}$	1.25	4.24	5.29
IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	3.58	$1.8x10^{3}$	1.26	4.24	5.35
IL4R	Interleukin 4 receptor	2.50	$1.7x10^{02}$	1.21	5.42	6.54
OR51B4	Olfactory receptor, family 51, subfamily B, member 4	2.43	$6.0x10^{3}$	1.23	4.25	5.22
OXTR	Oxytocin receptor	2.29	$2.8x10^{2}$	1.31	5.90	7.70
PDGFA	Platelet-derived growth factor α polypeptide	2.29	$3.6x10^{2}$	1.26	6.68	8.43
PTPN22	Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	2.79	$1.3x10^{2}$	1.29	3.60	4.65
RGS10	Regulator of G-protein signaling 10	2.96	$5.8x10^{3}$	1.19	8.25	9.83
STYK1	Serine/threonine/tyrosine kinase 1	2.25	$1.9x10^{2}$	1.25	4.15	5.17
STC2	Stanniocalcin 2	2.12	$4.9x10^{2}$	1.23	7.08	8.68
T-cell mediated immunity						
FOSL1	FOS-like antigen 1	2.36	$3.2x10^{2}$	1.25	7.99	9.99
IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	3.58	$1.8x10^{3}$	1.26	4.24	5.35
HLA-DMA	Major histocompatibility complex, class II, DM α	2.32	$3.4x10^{2}$	1.34	5.69	7.66
HLA-DRB1	Major histocompatibility complex, class II, DR β 1	2.18	$4.5x10^{2}$	1.35	5.99	8.08
HLA-DQB1	Major histocompatibility complex, class II, DQ β 1	2.22	$3.6x10^{2}$	1.26	5.16	6.49
Ligand-mediated signaling						
ADRB2	Adrenoceptor β 2, surface	3.28	$4.2 x10^{3}$	1.34	5.85	7.85
AHRR	Aryl-hydrocarbon receptor repressor	2.06	$5.5 x10^{2}$	1.25	6.24	7.83
FGF1	Fibroblast growth factor 1 (acidic)	1.37	$1.9x10^{1}$	1.03	3.89	4.00
IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte Maturation factor 1, p35)	3.58	$1.8x10^{3}$	1.26	4.24	5.35
IL4R	Interleukin 4 receptor	2.50	$1.7x10^{2}$	1.21	5.42	6.54
PDGFA	Platelet-derived growth factor α polypeptide	2.29	$3.6x10^{2}$	1.26	6.68	8.43
WNT5B	Wingless-type MMTV integration site family, member 5B	3.11	$7.4x10^{3}$	1.32	5.19	6.84

Table VII. Continued.

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

Table VII. Continued.

Gene	Description	Score	P-value	Fold-change	Up[a] mean	Down[a] mean
MGLL	Monoglyceride lipase	3.50	$2.6x10^{3}$	1.27	7.44	9.49
CYP2R1	Cytochrome P450, family 2, subfamily R, polypeptide	2.47	$2.5x10^{2}$	1.30	6.37	8.25
C11orf41	1 KIAA1549-like	2.16	$4.2x10^{2}$	1.21	4.64	5.62
LOC389906	Zinc finger protein 839 pseudogene	2.01	$6.2x10^{2}$	1.36	5.07	6.89
ATP8B1	ATPase, aminophospholipid transporter, class I, type 8B, member 1	2.75	$1.7x10^{2}$	1.35	6.06	8.21
EXT1	Exostosin glycosyltransferase 1	3.64	$1.4x10^{3}$	1.20	9.00	10.82
APCDD1L	Adenomatosis polyposis coli downregulated 1-like	2.30	$3.5x10^{2}$	1.27	5.46	6.92
LOC100506325	Uncharacterized LOC100506325	4.38	$1.2x10^{3}$	1.26	4.92	6.20
MCM3AP-AS1	MCM3AP antisense RNA 1	3.24	$6.6x10^{3}$	1.20	5.13	6.14
C10orf47	Proline and serine-rich protein 2	3.44	$4.8x10^{3}$	1.52	4.17	6.34
AFAP1L2	Actin filament associated protein 1-like 2	2.78	$1.3x10^{2}$	1.43	4.34	6.21
PARP8	Poly (ADP-ribose) polymerase family, member 8	2.52	$2.3x10^{2}$	1.22	5.07	6.20
UGT8	UDP glycosyltransferase 8	2.18	$4.3x10^{2}$	1.28	5.39	6.91
LOC730755	LOC730755	2.39	$3.2x10^{3}$	1.55	4.44	6.87
HBE1	Hemoglobin, epsilon 1	2.56	$1.7x10^{2}$	1.48	4.71	6.98
MPP4	Membrane protein, palmitoylated 4 (MAGUK p55 subfamily member 4)	2.89	$1.6x10^{3}$	1.42	3.50	4.96
CSTA	Cystatin A (stefin A)	2.04	$4.5x10^{2}$	1.39	3.80	5.29
SRGN	Serglycin	2.40	$2.7x10^{2}$	1.45	7.05	10.24
LOC100506465	Uncharacterized LOC100506465	2.55	$1.7x10^{2}$	1.30	4.40	5.72
MOK	MOK protein kinase	2.00	$5.8x10^{2}$	1.21	6.81	8.25
INPP4B	Inositol polyphosphate-4-phosphatase, type II, 105 kDa	2.59	$2.0x10^{2}$	1.37	5.53	7.60
AFAP1L1	Actin filament associated protein 1-like 1	2.21	$3.9x10^{2}$	1.23	4.77	5.87
CCBE1	Collagen and calcium binding EGF domains 1	2.07	$5.5x10^{2}$	1.37	4.55	6.25
KCNK1	Potassium channel, subfamily K, member 1	1.49	$2.2x10^{1}$	1.22	3.76	4.61
CCND2	Cyclin D2	2.31	$1.5x10^{2}$	1.35	3.98	5.36
CDA	Cytidine deaminase	1.43	$1.6x10^{1}$	1.04	7.58	7.86
DMKN	Dermokine	2.03	$5.7x10^{2}$	1.36	4.52	6.16
NOG	Noggin	2.06	$5.1x10^{2}$	1.44	4.04	5.82
GTSF1	Gametocyte specific factor 1	2.02	$6.6x10^{2}$	1.59	4.07	6.47
NT5E	5'-nucleotidase, ecto (CD73)	2.73	$1.2x10^{2}$	1.24	8.11	10.04
BIRC3	Baculoviral IAP repeat containing 3	2.07	$5.4x10^{2}$	1.24	4.88	6.05
NAP1L2	Nucleosome assembly protein 1-like 2	2.47	$2.3x10^{2}$	1.31	4.87	6.36
SLCO4A1	Solute carrier organic anion transporter family, member 4A1	2.39	$3.1x10^{2}$	1.30	6.35	8.26
KIAA1324L	KIAA1324-like	2.14	$4.9x10^{2}$	1.19	4.75	5.66
CYP2J2	Cytochrome P450, family 2, subfamily J, polypeptide 2	3.00	$8.8x10^{3}$	1.28	4.13	5.27
TUBA3C	Tubulin, α3c	2.44	$2.7x10^{2}$	1.20	5.59	6.70
CTAG2	Cancer/testis antigen 2	2.08	$7.2x10^{2}$	1.35	3.85	5.21
GALNTL4	UDP-N-acetyl-α-D-galactosamine: polypeptide-N-acetylgalactosaminyltransferase 18	2.52	$2.2x10^{02}$	1.26	5.66	7.10
MGC16121	MIR503 host gene (non-protein coding)	2.81	$1.2x10^{2}$	1.25	5.74	7.18
COL3A1	Collagen, type III, α1	2.32	$3.3x10^{2}$	1.53	5.05	7.74
PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2	1.98	$6.9x10^{2}$	1.25	7.17	8.98
BDNF-AS1	BDNF antisense RNA	2.91	$9.6x10^{3}$	1.25	4.12	5.16
KRTAP1-5	Keratin associated protein 1-5	2.40	$2.6x10^{3}$	1.37	4.06	5.55
CCDC80	Coiled-coil domain containing 80	2.54	$2.2x10^{2}$	1.29	6.78	8.73
NAP1L3	Nucleosome assembly protein 1-like 3	2.06	$5.6x10^{2}$	1.29	5.73	7.39

Table VII. Continued.

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

[a]Up, and down mean refers to the mean of the specific gene expression levels in the ten most PGC1a up- or downregulated cell lines. MHC, Major histocompatibility complex.

Table VIII. Annotated summary of class B of peroxisome proliferator-activated receptor γ, coactivator 1α.

Functional role	Genes	P-value	-Log (P-value)
Biological process			
MHCII-mediated immunity	3	$8.20x10^3$	2.09
Signal transduction	27	$9.70x10^3$	2.01
Intracellular signaling cascade	11	$1.10x10^2$	1.96
Cell surface receptor mediated signal transduction	16	$1.40x10^2$	1.85
T-cell mediated immunity	5	$1.40x10^2$	1.85
Ligand-mediated signaling	7	$1.60x10^2$	1.80
Calcium mediated signaling	4	$2.00x10^2$	1.70
Other oncogenesis	3	$4.30x10^2$	1.37
Cell communication	11	$6.90x10^2$	1.16
Cellular component			
MHC protein complex	4	$2.90x10^3$	2.54
Extracellular matrix	7	$7.80x10^3$	2.11
Extracellular region part	12	$8.40x10^3$	2.08
MHC class II protein complex	3	$9.30x10^3$	2.03
Extracellular region	18	$2.10x10^2$	1.68
Proteinaceous extracellular matrix	6	$2.30x10^2$	1.64
Apical plasma membrane	4	$2.90x10^2$	1.54
Chromatin assembly complex	2	$3.00x10^2$	1.52
Microsome	5	$3.20x10^2$	1.49
Vesicular fraction	5	$3.50x10^2$	1.46
Apical part of cell	4	$6.10x10^2$	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 *PGC1α* 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 *PGC1α*. *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 PGC1α 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 PGC1α

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

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

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 ($P<0.05$). [a]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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References

1. Finck BN and Kelly DP: PGC-1 coactivators: Inducible regulators of energy metabolism in health and disease. J Clin Invest 116: 615-622, 2006.
2. Knutti D and Kralli A: PGC-1, a versatile coactivator. Trends Endocrinol Metab 12: 360-365, 2001.
3. Scarpulla RC: Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. Biochim Biophys Acta 1813: 1269-1278, 2011.
4. Puigserver P and Spiegelman BM: Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): Transcriptional coactivator and metabolic regulator. Endocr Rev 24: 78-90, 2003.
5. Lin J, Handschin C and Spiegelman BM: Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1: 361-370, 2005.
6. Tritos NA, Mastaitis JW, Kokkotou EG, Puigserver P, Spiegelman BM and Maratos-Flier E: Characterization of the peroxisome proliferator activated receptor coactivator 1 alpha (PGC 1alpha) expression in the murine brain. Brain Res 961: 255-260, 2003.
7. Cowell RM, Blake KR and Russell JW: Localization of the transcriptional coactivator PGC-1alpha to GABAergic neurons during maturation of the rat brain. J Comp Neurol 502: 1-18, 2007.
8. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, Handschin C, Zheng K, Lin J, Yang W, *et al*: Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell 127: 397-408, 2006.
9. Watkins G, Douglas-Jones A, Mansel RE and Jiang WG: The localisation and reduction of nuclear staining of PPARgamma and PGC-1 in human breast cancer. Oncol Rep 12: 483-488, 2004.
10. Feilchenfeldt J, Brüundler MA, Soravia C, Tötsch M and Meier CA: Peroxisome proliferator-activated receptors (PPARs) and associated transcription factors in colon cancer: Reduced expression of PPARgamma-coactivator 1 (PGC-1). Cancer lett 203: 25-33, 2004.
11. Jiang WG, Douglas-Jones A and Mansel RE: Expression of peroxisome-proliferator activated receptor-gamma (PPARgamma) and the PPARgamma co-activator, PGC-1, in human breast cancer correlates with clinical outcomes. Int J Cancer 106: 752-757, 2003.
12. Zhang Y, Ba Y, Liu C, Sun G, Ding L, Gao S, Hao J, Yu Z, Zhang J, Zen K, *et al*: PGC-1alpha induces apoptosis in human epithelial ovarian cancer cells through a PPARgamma-dependent pathway. Cell Res 17: 363-373, 2007.
13. Vander Heiden MG, Cantley LC and Thompson CB: Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science 324: 1029-1033, 2009.
14. Vazquez F, Lim JH, Chim H, Bhalla K, Girnun G, Pierce K, Clish CB, Granter SR, Widlund HR, Spiegelman BM and Puigserver P: PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. Cancer cell 23: 287-301, 2013.
15. Plate KH and Risau W: Angiogenesis in malignant gliomas. Glia 15: 339-347, 1995.
16. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, *et al*: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987-996, 2005.
17. Cancer Genome Atlas Research Network: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455: 1061-1068, 2008.
18. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, *et al*: Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17: 98-110, 2010.
19. Lee YS, Kang JW, Lee YH and Kim DW: ID4 mediates proliferation of astrocytes after excitotoxic damage in the mouse hippocampus. Anat Cell Biol 44: 128-134, 2011.
20. Yi MH, Kim S, Zhang E, Kang JW, Park JB, Lee YH, Chung CK, Kim YM and Kim DW: IQGAP1 expression in spared CA1 neurons after an excitotoxic lesion in the mouse hippocampus. Cell Mol Neurobiol 33: 1003-1012, 2013.

21. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehár J, Kryukov GV, Sonkin D, *et al*: The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 483: 603-607, 2012.
22. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, *et al*: Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring. Science 286: 531-537, 1999.
23. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC and Lempicki RA: The DAVID gene functional classification tool: A novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 8: R183, 2007.
24. Vlasblom J, Zuberi K, Rodriguez H, Arnold R, Gagarinova A, Deineko V, Kumar A, Leung E, Rizzolo K, Samanfar B, *et al*: Novel function discovery with GeneMANIA: A new integrated resource for gene function prediction in Escherichia coli. Bioinformatics 31: 306-310, 2015.
25. Meng H, Liang HL and Wong-Riley M: Quantitative immuno-electron microscopic analysis of depolarization-induced expression of PGC-1alpha in cultured rat visual cortical neurons. Brain Res 1175: 10-16, 2007.
26. Deighton RF, Le Bihan T, Martin SF, Gerth AM, McCulloch M, Edgar JM, Kerr LE, Whittle IR and McCulloch J: Interactions among mitochondrial proteins altered in glioblastoma. J Neurooncol 118: 247-256, 2014.
27. Soyal SM, Felder TK, Auer S, Hahne P, Oberkofler H, Witting A, Paulmichl M, Landwehrmeyer GB, Weydt P and Patsch W; European Huntington Disease Network: A greatly extended PPARGC1A genomic locus encodes several new brain-specific isoforms and influences Huntington disease age of onset. Hum Mol Genet 21: 3461-3473, 2012.
28. Choi J, Batchu VV, Schubert M, Castellani RJ and Russell JW: A novel PGC-1α isoform in brain localizes to mitochondria and associates with PINK1 and VDAC. Biochem Biophys Res Commun 435: 671-677, 2013.
29. Chapman EJ and Knowles MA: Necdin: A multi functional protein with potential tumor suppressor role? Mol Carcinog 48: 975-981, 2009.
30. Fujiwara K, Hasegawa K, Ohkumo T, Miyoshi H, Tseng YH and Yoshikawa K: Necdin controls proliferation of white adipocyte progenitor cells. PloS One 7: e30948, 2012.
31. Niinobe M, Koyama K and Yoshikawa K: Cellular and subcellular localization of necdin in fetal and adult mouse brain. Dev Neurosci 22: 310-319, 2000.