PLOS ONE

RESEARCH ARTICLE

Oncometabolic role of mitochondrial sirtuins in glioma patients

Maria Fazal Ul Haq[1☯], **Muhammad Zahid Hussain**[2], **Ishrat Mahjabeen**[1☯]*,
Zertashia Akram[1], **Nadia Saeed**[1], **Rabia Shafique**[1], **Sumaira Fida Abbasi**[1], **Mahmood Akhtar Kayani**[1]

1 Cancer Genetics and Epigenetics Research Group, Department of Biosciences, COMSATS University Islamabad, Islamabad, Pakistan, 2 Department of Rheumatology, Military hospital, Rawalpindi, Pakistan

☯ These authors contributed equally to this work.
* ishrat.mahjabeen@comsats.edu.pk

Abstract

Mitochondrial sirtuins have diverse role specifically in aging, metabolism and cancer. In cancer, these sirtuins play dichotomous role as tumor suppressor and promoter. Previous studies have reported the involvement of sirtuins in different cancers. However, till now no study has been published with respect to mitochondrial sirtuins and glioma risks. Present study was purposed to figure out the expression level of mitochondrial sirtuins (*SIRT3*, *SIRT4*, *SIRT5*) and related genes (*GDH*, *OGG1-2α*, *SOD1*, *SOD2*, *HIF1α* and *PARP1*) in 153 glioma tissue samples and 200 brain tissue samples from epilepsy patients (taken as controls). To understand the role of selected situins in gliomagenesis, DNA damage was measured using the comet assay and oncometabolic role (oxidative stress level, ATP level and NAD level) was measured using the ELISA and quantitative PCR. Results analysis showed significant down-regulation of *SIRT4* (p = 0.0337), *SIRT5* (p<0.0001), *GDH* (p = 0.0305), *OGG1-2α* (p = 0.0001), *SOD1* (p<0.0001) and *SOD2* (p<0.0001) in glioma patients compared to controls. In case of *SIRT3* (p = 0.0322), *HIF1α* (p = 0.0385) and *PARP1* (p = 0.0203), significant up-regulation was observed. ROC curve analysis and cox regression analysis showed the good diagnostic and prognostic value of mitochondrial sirtuins in glioma patients. Oncometabolic rate assessment analysis showed significant increased ATP level (p<0.0001), NAD+ level [(NMNAT1 (p<0.0001), NMNAT3 (p<0.0001) and NAMPT (p<0.04)] and glutathione level (p<0.0001) in glioma patients compared to controls. Significant increased level of damage ((p<0.04) and decrease level of antioxidant enzymes include superoxide dismutase (SOD, p<0.0001), catalase (CAT, p<0.0001) and glutathione peroxidase (GPx, p<0.0001) was observed in patients compared to controls. Present study data suggest that variation in expression pattern of mitochondrial sirtuins and increased metabolic rate may have diagnostic and prognostic significance in glioma patients.

Introduction

Gliomas are among the most common type of CNS tumors and epidemiological studies have stated that gliomas are 40% of all CNS and brain tumors [1–4]. On the basis of molecular and

histopathological features, gliomas are classified into low grade gliomas (LGG) and high-grade gliomas (HGG). Risk factors of gliomas are environmental factors, epigenetic and genetic factors. Genetic factors include the genetic variations/polymorphisms in metabolic/repairing pathway genes, apoptosis, and necroptosis [5, 6]. Most of the data published on genetic factors is present on mutations in nuclear region while the involvement of mitochondrial genes in glioma is lacking [5].

Mitochondria is very important organelle for cellular processes like energy production by oxidative phosphorylation, apoptosis, cell cycle proliferation etc. Reactive oxygen species (ROS) are highly produced in mitochondria and when this ROS levels are high, they can be harmful to cell, damages proteins, lipids and DNA hence results in mitochondrial dysfunction and carcinogenesis [7]. Brain and CNS consume a high percentage of energy and neurons are more susceptible to oxidative damage and ROS and has been found to be involved in neurodegenerative diseases [8]. Rate of mutation has been reported higher in mitochondrial DNA as it lacks the protective histone proteins and inefficient DNA repair mechanisms [9]. Furthermore, in glioma patients, another mitochondrial mechanism actively involved is necroptosis, which controlled the deep dyregulation of apoptosis signals. This dyregulation of programmed cell death ultimately results in gliomagenesis and tumor aggressiveness [6, 10].

Mitochondrial variations have also been found to link with carcinogenesis by deregulation of number of important mechanisms [11]. Among these pathways, variations of mitochondrial sirtuins genes have been suggested. Sirtuins, a family of orthologues of yeast silent information regulator 3 (*SIRT3*), 4 (*SIRT4*) and 5 (*SIRT5*) are important tumor suppressor genes located in mitochondria [12, 13]. Among these proteins, *SIRT3* is considered as guardian of mitochondria due to its role in deacetylation and metabolism [14]. Its deacetylation results in activation of proteins essential to control the oxidative stress and DNA damage/repair [15]. *SIRT4*, second mitochondrial sirtuin, has been reported to control the generation of reactive oxygen species (ROS) in mitochondria. Furthermore, it is actively involved in an NAD+ dependent protein ADP-ribosly transferase activity [16]. *SIRT5*, third mitochondrial sirtuin, has been reported as a mitochondrial NAD+ dependent deacetylase and involved in ROS detoxification and TCA cycle [17]. Previous studies have reported the involvement of mitochondrial sirtuins in regulation of important mitochondrial pathways such as ROS detoxification, repair, apoptosis/necroptosis and energy metabolism [18–20]. Mitochondrial sirtuins showed their involvement in above mentioned physiological processes in coordination with other genes. Kumar and Lombard (2015) has reported that molecular targets of mitochondrial sirtuin *SIRT3*, *SIRT4* and *SIRT5* have been identified in proteomic experiments [21]. These targets are such as *hypoxia inducible factor 1 subunit alpha (HIF1a)*, *glutamate dehydrogenase* (*GDH*), *Superoxide dismutase 1* (*SOD1*), *Superoxide dismutase 2 (*SOD2*), mitochondrial *8-oxoguanine DNA glycosylase* (*OGG1-2α*) and *poly (ADP-ribose) polymerase 1 (PARP1)* [13]. Mitochondrial sirtuins regulate the mitochondria ROS level in coordination with *SOD1*, *SOD2*, *HIF1a* and *OGG1-2α* and any abnormality in this regulation will results initiation of carcinogenesis [13, 22–29]. Mitochondrial sirtuins also controls the other molecules by deacetylation such as it deacetylates the *GDH* and maintained its regulatory role in mitochondrial metabolism [28]. Previous studies have reported that mitochondrial sirtuins interact with the *PARP1* and control the regulation of DNA repairing, cell survival and cell death [30].

Several studies have reported the role of mitochondrial sirtuins and above-mentioned genes in different diseases [22–29]. However, no study has been yet published with respect to glioma. Thus, the current study is designed to investigate expression level of *SIRT3*, *SIRT4*, *SIRT5* and associated genes *SOD1*, *SOD2*, *OGG1-2α*, *PARP1*, *GDH* and *HIF1α* in glioma patients and to find out whether this expressional deregulation effects the risk of glioma. Metabolic assays and DNA damage will also be performed to assess the ATP, NAD+, glutathione and oxidative stress levels in glioma samples.

Methodology

Patients identification and sampling

Glioma patients were identified with the help of oncologists. Samples of different types and grades were collected by following the CNS WHO 2016 grading system. Study cohort included 153 glioma tissue samples from Pakistan Institute of Medical Sciences. In case of controls, 200 surgical section of brain tissue of epilepsy patients was collected from neurosurgical section of the above-mentioned hospital. Epilepsy patients are taken as controls as per guidelines of previously reported studied of brain tumoriogenesis [31, 32]. Demographic details of study cohort are given in Table 1. The surgically excised tissues of glioma patients and controls were recruited and stored at -80˚C. Histopathological reports for each sample was also obtained. Other factors like smoking, ethnicity, age, gender was recorded to link with disease.

Ethical approval

The study was conducted with a prior approval from the institutional ethical review board of COMSTAS University (CUI) Islamabad. Members of this committee include Dean ORIC (Office of Research Innovation and Commercialization) Prof. Dr. Mahmood A Kayani (convener), Professor Dr Mustafa (Chairman Deptt of Biosciences), Dr. Faheem Tahir (Deputy Director, NIH) and Dr. Muhammad Qaiser Fatmi (Head of department). Informed written/ signed consent was obtained from participating individuals.

RNA extraction and cDNA synthesis. RNA was extracted from the fresh tissue samples by Trizol method as previously described by Afzal et al., [33]. Extracted RNA was visualized and quantified by the 1% agarose gel electrophoresis and spectrophotometer. RNA samples with 50 ng concentration were selected and proceeded for cDNA synthesis using the commercially available kit by Thermo Scientific (USA). Synthesized cDNA was confirmed by *β-actin* PCR and stored at -20˚C until further processing.

Table 1. Demographic parameters of study cohort.

Parameters	Glioma patients N = 153	Controls N = 200
Age		
<41	55	47
>41	98	153
Gender		
Males	69	98
Females	84	102
WHO grade		
I	15	
II	69	
III	32	
IV	37	
Survival Status		
Under treatment/ cured	102	
Dead	51	
Smoking Status		
Smokers	86	
Non- smokers	67	
IR Exposure		
Yes	59	
No	94	

Expression analysis

Primers specific for the selected genes *SIRT3, SIRT4, SIRT5, SOD1, SOD2, OGG1-2α, PARP1, GDH, HIF1α* and *β-actin* was designed using the integrated DNA technology (IDT) as previously described by Ul Haq et al., [16]. Expression levels of above mentioned genes were measured using the Quantitative real time polymerase chain reaction (q-RT PCR). Step one Plus Thermal cycler (Applied Biosystems) was used for this analysis. Each reaction constituted of 4 μl SYBR green, 2 μl of primers, 1 μl cDNA and 3 μl of PCR water. q-RT PCR conditions included, 10 min of initial denaturation, 1 min of annealing for 40 cycles. Cyclic threshold (CT) values were taken for each sample and $2^{-\Delta\Delta Ct}$ method was used to calculate relative expression.

Validation by NCBI GEO omnibus dataset

Involvement of these genes was assessed in genome wide expression in one dataset. The dataset containing glioma samples along with control samples was selected. The publicly available data to compare with our PCR results was obtained from NCBI Gene Expression Omnibus (GEO) database, (https://www.ncbi.nlm.nih.gov/geo/). Experiment type was Expression profiling by array with Accession number GSE:4290, Platform GPL570 (HG-U133_plus_2) Affymetrix Human Genome U133 Plus 2.0 Array [16].

Comet assay

Single cell gel electrophoresis was carried out for the estimation of DNA damage in glioma patients and healthy controls. Procedure adopted was carried out from Akram et al., [34] with some modifications. Glioma tissue samples of different subtypes along with healthy control tissues were used. The tissues were homogenized in saline solution and single cell suspension was prepared which was proceeded for detection of overall DNA damage in the tissues. Glass slides used in the procedure were labelled according to different glioma types. Three layered procedure was used the samples were sandwiched in the layers of low melting point (LMP) agarose gel. Whole area under coverslip was visualized and scored by fluorescent microscope. After running, mean %DNA damage, mean OTM (olive tail moment) and mean tail length were obtained and further processed for analysis. Comets were analyzed in Metafer 4 (MetaSystems).

Metabolic assays

ATP level determination. Glioma tissue sample and controls taken from patients were treated with saline solution to prepare tissue lysate. Three aliquots were prepared, one aliquot was used for the measurement of ATP level determination, as described by Afzal et al., [33]. Second aliquot was used for oxidative stress measurement and third for DNA damage assessment. For ATP level measurement, cell lysate of glioma and healthy controls were dispensed in micro titer plate and Cell titer glo (CTG) buffer was then added in the micro titer plate and Luminescence was recorded and analyzed.

Oxidative stress determination. Glutathione level was measured from isolated protein lysate using Glutathione Assay Kit II (Calbiochem) according to manufacturer protocol provided with kit, as described by Afzal et al., [33].

Oxidative stress was measured by the cell lysate of the glioma samples and healthy controls. Oxidative stress was evaluated by quantitative analysis of three antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Enzyme analysis was carried out using human specific ELISA kits purchased from Abcam (UK).

Absorbency of enzymes was checked in microplate reader (Platos R496, AMP Diagnostics), and sample concentrations were read from calibration curve.

NAD level determination. NAD level was also measured by designing the primers for three important enzymes regulating NAD levels, they are NAMPT, NMNAT1 and NMNAT3. The q-RT-PCR procedure and program designing was same as stated above for expression analysis.

Data analysis. Statistical tools from SPSS and GraphPad Prism were used to analyze the data. *SIRT3*, *SIRT4*, *SIRT5*, *SOD1*, *SOD2*, *GDH*, *mt OGG1-2α*, *PARP1* and *HIF1a* expression values were compared using student t-test, DNA damage values were compared using student t-test and one sample t-test while NAD+ and ATP values were also compared using student t-test. Gene to gene and gene to different histopathological parameters were compared by applying spearman correlation and diagnostic value was checked by ROC analysis. Survival analysis was performed by Kaplan Maier analysis.

Results

Expressional analysis

Relative expression of these selected genes was analyzed in 153 glioma samples along with 200 controls using qPCR. Significant down-regulated expression of *SIRT4* (p = 0.033), *SIRT5* (p<0.0001), *GDH* (p = 0.03), *OGG1-2α* (p<0.0001), *SOD1* (p<0.0001) and *SOD2* (p<0.0001) was observed in glioma patients compared to controls as shown in Fig 1A and 1C. In case of *SIRT3* (p = 0.03), *HIF1α* (p = 0.03) and *PARP1* (p = 0.02) gene, significant upregulated expression was observed in glioma patients compared to control samples as shown in Fig 1A and 1C.

Demographic details of selected glioma patients and controls are given in Table 1. In case of grading, significant upregulation of *SIRT3* (p = 0.0128) was observed in high grade glioma (HGG) compared to low grade glioma (LGG) as shown in Fig 1A. All other genes such as *SIRT4* (p = 0.0129; Fig 2B), *SIRT5* (p = 0.0461; Fig 2C), *SOD1* (p = 0.0239; Fig 2D), *SOD2* (p = 0.0066; Fig 2E), *OGG1-2α* (p = 0.0096, Fig 2F), *HIF1α* (p = 0.0149; Fig 2G), *GDH* (p = 0.0469; Fig 2H) and *PARP1* (p = 0.0087; Fig 2I) were significantly downregulated in HGGs as compared to LGG. In case of smoking status, *SIRT3* was found significantly upregulated (p = 0.0097; Fig 1A) and *SIRT4* (p = 0.0015; Fig 2B), *OGG1-2α* (p = 0.0026; Fig 2F), *SOD1* (p = 0.0445; Fig 2D) and *SOD2* (p = 0.0267; Fig 2E) were significantly downregulated in smokers compared to non-smokers. While non-significant results were observed in case of *SIRT5* (p = 0.3604; Fig 2C), *GDH* (p = 0.204; Fig 2H), *HIF1α* (p = 0.1500; Fig 2G) and *PARP1* (p = 0.4626; Fig 2I) in smokers compared to nonsmokers.

Validation by NCBI GEO Omnibus dataset. Relative expression of our selected genes was validated from dataset GSE4290, titled: Expression data of glioma from Henry Ford hospital, at first data was normalized and analyzed in GraphPad Prism. Data set consisted of 157 glioma samples and 23 Epilepsy samples which were used as controls. 157 tumor samples include 26 astrocytoma, 50 oligodendrogliomas and 81 glioblastomas. Data analysis showed that *SIRT4* (p<0.0001), *SIRT5* (p = 0.0076), *OGG1-2α* (p<0.0001), *SOD1* (p<0.0001) and *SOD2* (p<0.0001) showed significant downregulation as shown in Fig 1B and 1D. while *GDH* (p = 0.6776) showed non significantly downregulated results (Fig 1D). In case of *SIRT3* (p = 0.0142) and *HIF1α* (p = 0.0385) significant upregulation was observed (Fig 1B and 1D) while non-significant upregulated expression was observed in *PARP1* (p = 0.203) in glioma samples as compared to healthy samples. Results are shown in Fig 1B and 1D.
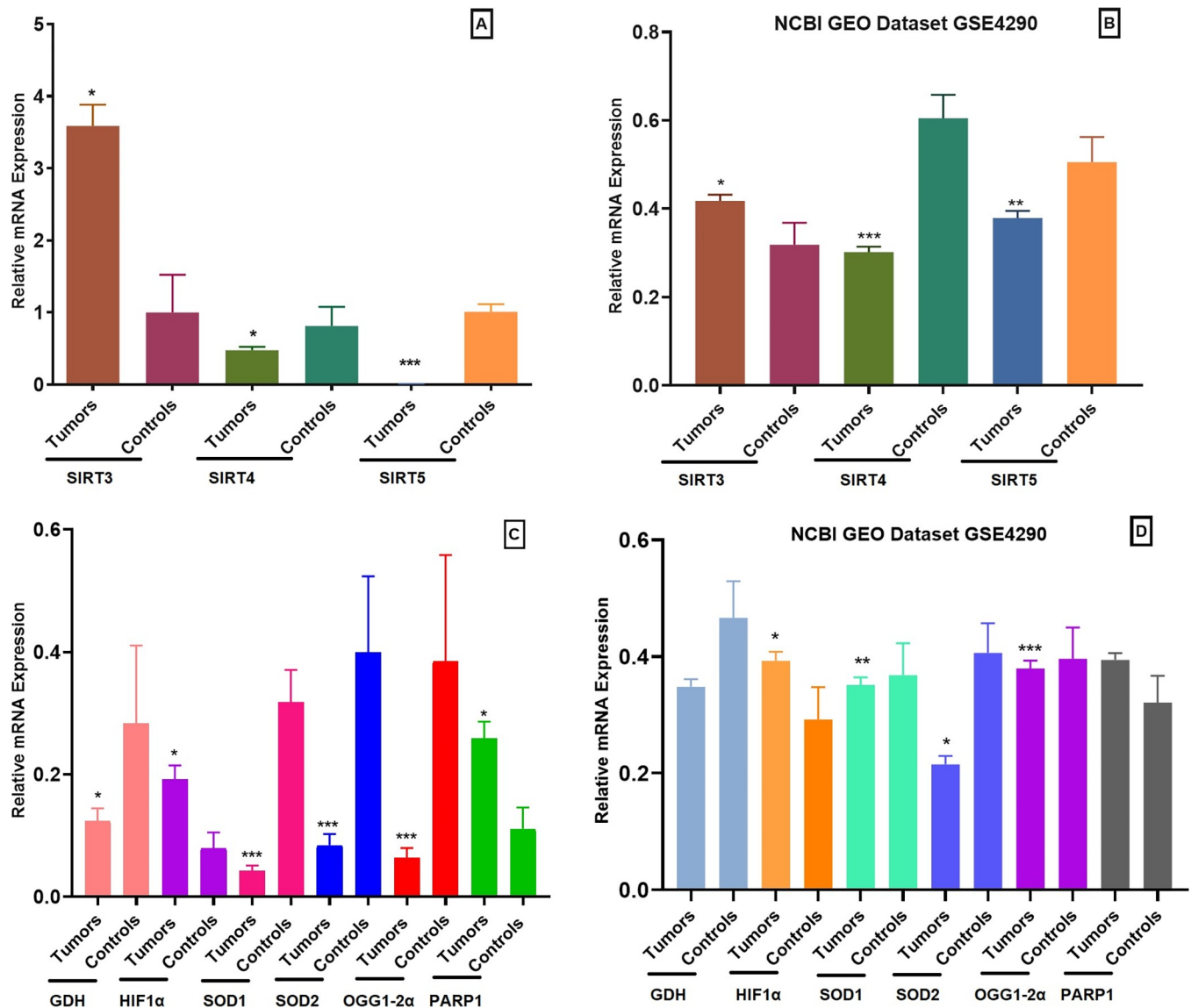
Fig 1. q-PCR analysis of mitochondrial sirtuins and relative genes in glioma patients compared to controls. (A) Relative expression levels of mitochondrial sirtuins *SIRT3*, *SIRT4* and *SIRT5* in glioma patients compared to controls. (B) Relative expression levels of mitochondrial sirtuins *SIRT3*, *SIRT4* and *SIRT5* in publicly available dataset of glioma tumors compared to controls. (C) Relative expression levels of mitochondrial sirtuins related genes such as *GDH*, *HIF1a*, *SOD1*, *SOD2*, *OGG1-2α* and *PARP1* in glioma patients compared to controls. (D) Relative expression levels of *GDH*, *HIF1a*, *SOD1*, *SOD2*, *OGG1-2a* and *PARP1* in publicly available dataset of glioma tumors compared to controls. $P<0.05^*$, $p<0.01^{**}$, $p<0.001^{***}$.

https://doi.org/10.1371/journal.pone.0281840.g001

Comet assay

For DNA damage assessment, comet assay was performed for glioma samples and uninvolved healthy controls. In this assay number of comets were assessed in glioma sections Fig 3A2 compared to the healthy controls Fig 3A1. Data analysis showed significantly higher number of comets in tumor samples (p = 0.04) compared to the controls (Fig 3B).

In the assay different parameters were also calculated which are %DNA in tail and it showed significant higher %DNA in tail in glioma patients compared to controls (p = 0.0005), HGG compared to LGG (p = 0.0374) and smokers in comparison with non-smokers (p = 0.0320) as shown in Fig 3C and 3F. In case of olive tail moment, it was observed significantly higher in
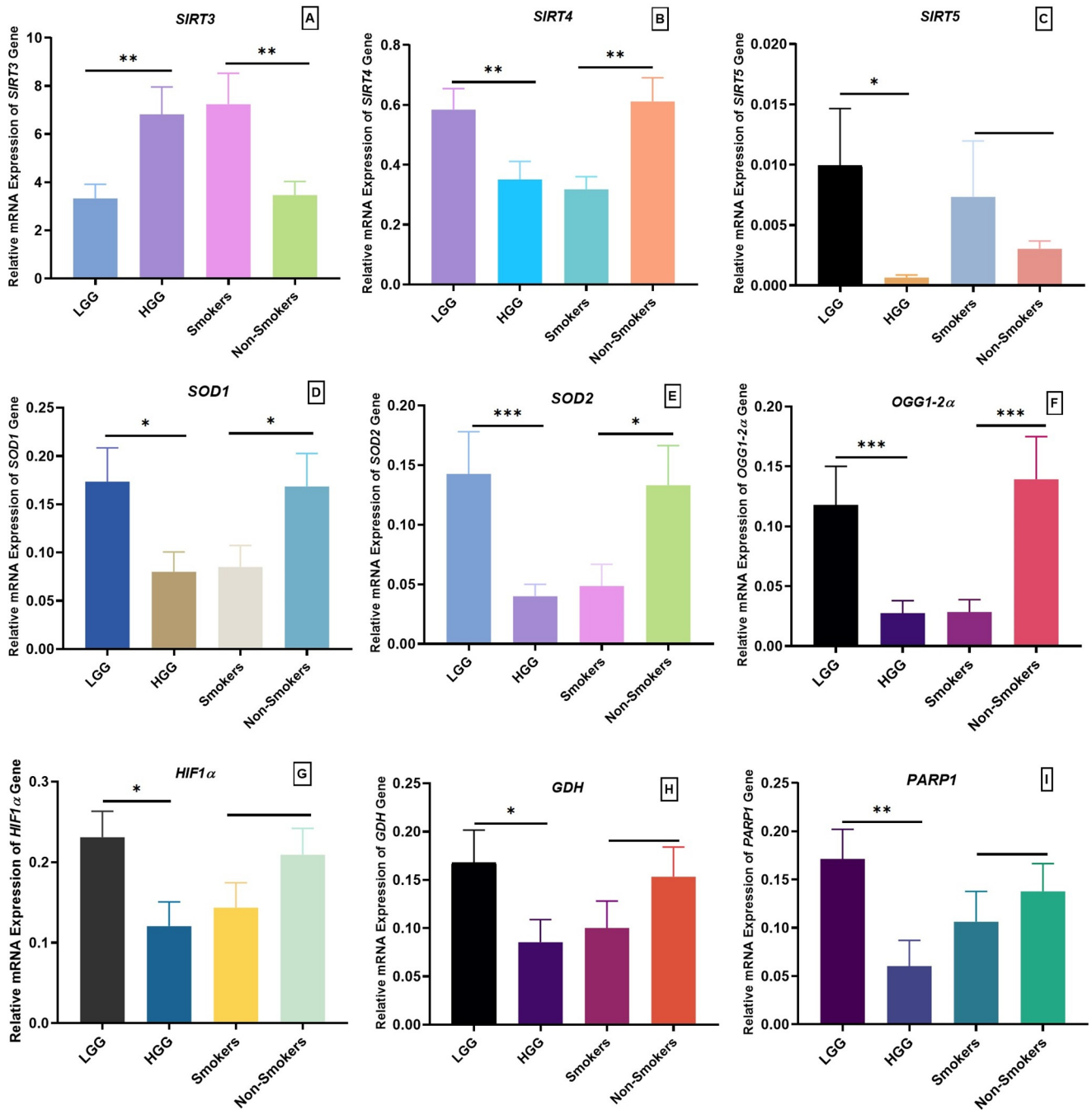
Fig 2. Association of relative expression of mitochondrial sirtuins and relative genes with histopathological parameters of glioma patients. (A) Relative expression of *SIRT3* gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (B) Relative expression of *SIRT4* gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (C) Relative expression of *SIRT5* gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (D) Relative expression of *SOD1* gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (E) Relative expression of *SOD2* gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (F) Relative expression of *OGG1-2a* gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (G) Relative expression of HIF1a gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (H) Relative expression of *GDH* gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (I) Relative expression of *PARP1* gene in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. $P < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$.
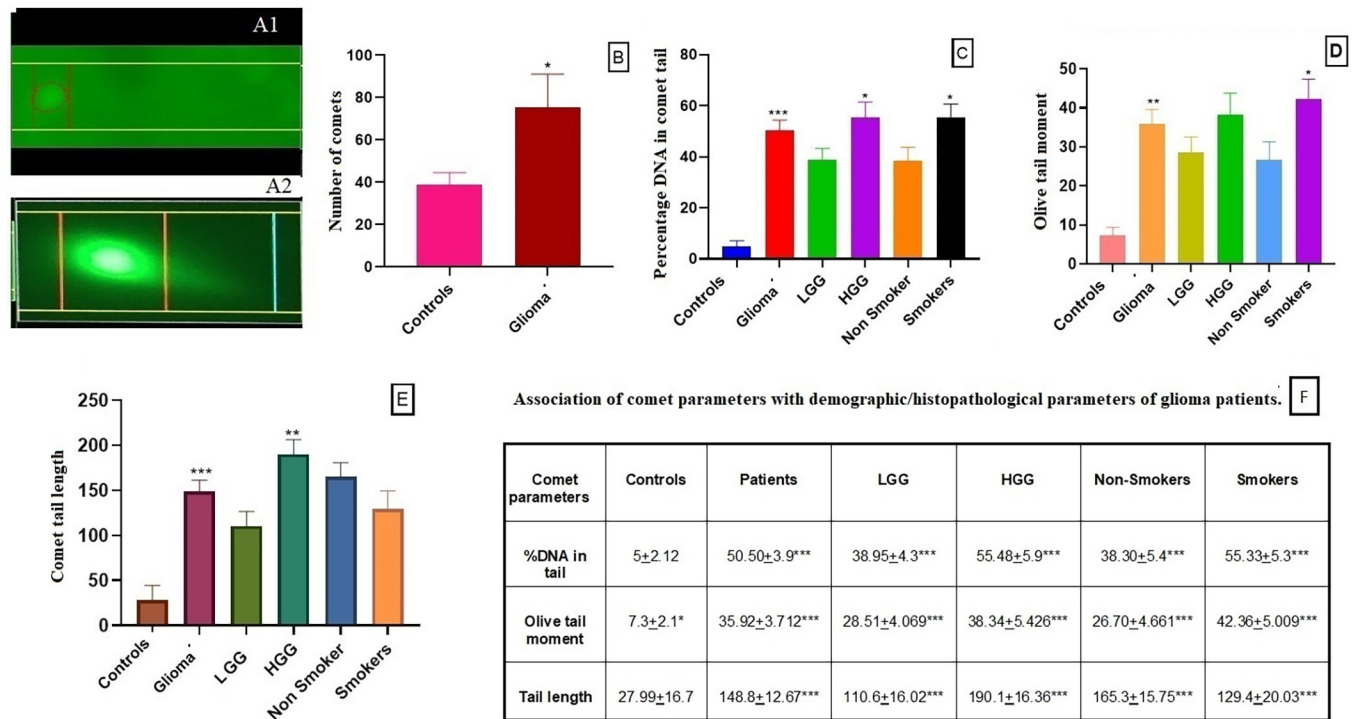
https://doi.org/10.1371/journal.pone.0281840.g002

Fig 3. Comet analysis of glioma patients and controls. (A1) Comet tail and length in controls, (A2) Comet tail and length in glioma, (B) number of comets in glioma patients vs controls, (C) percentage DNA in comet tail in glioma patients vs controls, with respect to different grades and smoking status of glioma patients, (D) olive tail moment in glioma patients vs controls, with respect to different grades and smoking status of glioma patients. (E) comet tail length in glioma patients vs controls, with respect to different grades and smoking status of glioma patients, (F) association of comets with different demographic/histopathological parameters of glioma patients.

https://doi.org/10.1371/journal.pone.0281840.g003

glioma tissue samples compared to controls ($p = 0.008$), smokers compared to non-smokers ($p = 0.0266$) and non-significantly higher in case of HGG as compared to LGG ($p = 0.1582$) as shown in Fig 3D and 3F. Tail length was also assessed in patients and controls, tail length was observed higher in glioma patients compared to controls ($p = 0.0005$), HGG compared to LGG ($p = 0.0011$) while non-significantly less in smokers as compared to non-smokers ($p = 0.1605$) as shown in Fig 3E and 3F, Table 2.

Metabolic assays

ATP level was determined in glioma samples compared to the healthy controls. Analysis showed that ATP level was found significantly higher ($p<0.03$) in glioma compared to the control samples as shown in Fig 4A. ATP levels were also found to be significantly higher in HGG as compared to LGG and non-significantly low in smokers as compared to non-smokers, as shown in Fig 4B.

Glutathione level was also assessed, and it was found to be significantly higher in glioma patients compared to controls ($p<0.0001$) and in HGG as compared to LGG ($p<0.001$), as shown in Fig 4C and 4D. While in case of smokers, glutathione level was found to be non-significantly higher as compared to non-smokers Fig 4D.

NAD+ level was also determined by measuring the relative expression levels of NMNAT1, NMNAT3 and NAMPT. NMNAT1 was found significantly upregulated in gliomas ($p<0.001$) compared to controls and in HGG ($p<0.001$) compared to LGG patients. While in case of smokers, NMNAT1 was found non significantly upregulated compared to non-smokers as

Table 2. Multivariant COX regression analysis of selected microRNAs in glioma patients.

Parameters	B	SE	Wald	DF	Sig	Exp (B)
SIRT3	1.724	0.524	10.827	1	0.0001	5.61
SIRT4	1.451	0.441	10.827	1	0.001	4.27
SIRT5	1.297	0.462	7.879	1	0.005	3.66
SOD1	0.190	0.112	2.874	1	0.09	1.21
SOD2	0.418	0.231	3.283	1	0.07	1.52
GDH	1.095	0.425	6.634	1	0.01	2.99
OGG1-2a	1.187	0.461	6.634	1	0.003	3.28
PARP1	1.515	0.460	10.827	1	0.001	4.55
HIF1α	1.795	0.461	15.136	1	0.0001	6.02
Age	0.212	0.310	0.467	1	0.495	1.236
Gender	-0.562	0.319	3.09	1	0.078	0.570
Smoking	-0.587	0.315	3.461	1	0.063	0.556
Grade	-0.628	0.318	3.908	1	0.04	0.534
IR exposure	-0.514	0.303	2.880	1	0.09	0.598

SE = standard error; DF = degree of freedom; Sig = significance $p<0.05$

shown in Fig 5A. Second selected gene, NMNAT3 was found significantly upregulated in gliomas ($p<0.001$) compared to controls and in HGG ($p<0.001$) compared to LGG as shown in Fig 5B. while in case of smokers, NMNAT3 was found non significantly upregulated compared to non-smokers Fig 5B. Third selected gene, NAMPT was found significantly upregulated in gliomas compared to controls ($p<0.04$) and in HGG as compared to LGG ($p<0.001$) as shown in Fig 5C. while in case of smokers NAMPT was found non significantly upregulated as compared to non-smokers as shown in Fig 5C.

Measurement of oxidative stress

Oxidative stress was evaluated in study cohort by measuring the three main antioxidant enzymes which includes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) as shown in Fig 6. SOD ($p<0.001$), CAT ($p<0.001$) and GPx ($p<0.001$) levels were found significantly down regulated in glioma patients compared to controls, as shown in Fig 6A. Further analysis showed the significant downregulated expression of SOD ($p<0.001$), CAT ($p<0.001$) and GPx ($p<0.001$) in HGG compared to LGG as shown in Fig 6B and in smokers [SOD ($p<0.001$), CAT ($p<0.02$) and GPx ($p<0.04$)] compared to non-smokers (Fig 6C).

Spearman correlation

Gene to gene correlation was performed by spearman mentioned in Table 3. Negative correlation was observed between *SIRT3* and *SIRT4* (r = -0.2213 p = 0.050), *SIRT3* and *SIRT5* (r = -0.04447 p = 0.697), *SIRT3* and *SOD2* (r = -0.03706 p = 0.746), *SIRT3* and *HIF1α* (r = -0.09681 p = 0.396), *SIRT4* and *SOD1* (r = -0.03940 p = 0.712), *SIRT4* and *GDH* (r = -0.1180 p = 0.268), *SIRT4* and *SOD1* (r = -0.03940 p = 0.712), *SIRT4* and *HIF1α* (r = -0.09176 p = 0.390), *SIRT5* and *SOD1* (r = -0.0529 p = 0.620), *SIRT5* and *HIF1α* (r = -0.01410 p = 0.89),*SIRT5* and *PARP1* (r = -0.0927 p = 0.89),*SIRT5* and *OGG1-2α* (r = -0.1080 p = 0.311), *SIRT5* and *SOD2* (r = -0.0456 p = 0.670), *SIRT5* and *GDH* (r = -0.01657 p = 0.877), *SOD1* and *GDH* (r = -0.2783, p = 0.015*), *GDH* and *HIF1a* (r = -0.04, p = 0.712), *GDH* and *PARP1* (r = -0.0606, p = 0.603),
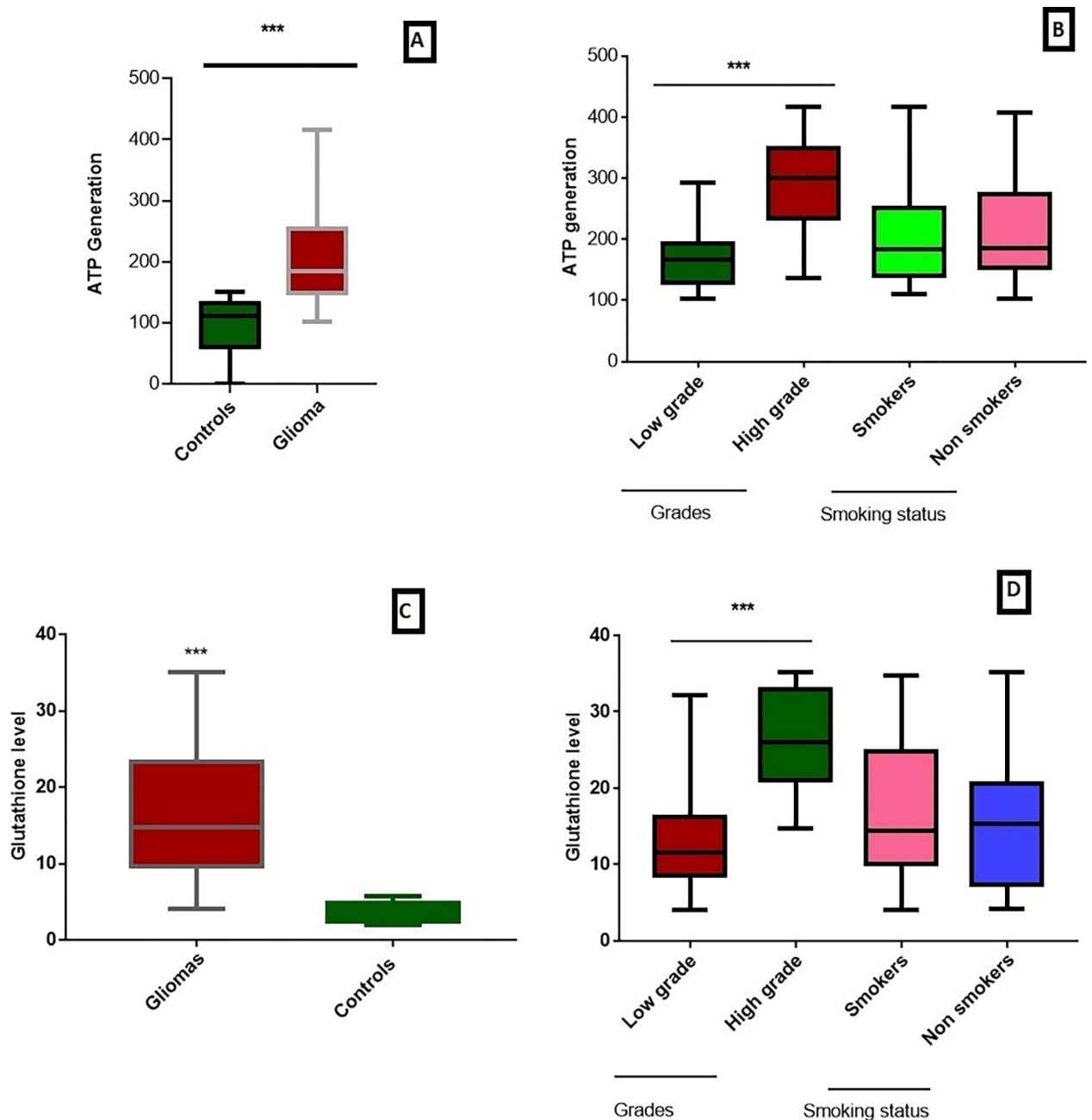
Fig 4. Measurement of ATP and glutathione levels in glioma vs controls. (A) ATP level in glioma vs controls. (B) ATP level in in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. (C) Glutathione level in glioma vs controls. (D) Glutathione level in in low grade glioma (LGG) vs high grade glioma (HGG) and in smokers vs nonsmokers. P<0.05*, p<0.01**, p<0.001***.

SOD1 and *HIF1a* (r = -0.079, p = 0.486), *SOD2* and *HIF1a* (r = -0.0149, p = 0.896), *SOD1* and *SOD2* (r = -0.0149, p = 0.483) in glioma patients.

Furthermore, positive association was observed between *SIRT3* and *SOD1* (r = 0.06661 p = 0.560), *SIRT3* and *PARP1* (r = 0.2825 p = 0.012*), *SIRT3* and *GDH* (r = 0.0067 p = 0.953), *SIRT3* and *OGG1-2α* (r = 0.2401 = 0.033*), *SIRT4* and *SIRT5* (r = 0.04925 p = 0.647), *SIRT4* and *PARP1* (r = 0.01022 p = 0.924), *SIRT4* and *SOD2* (r = 0.02412 p = 0.821), *SOD1* and
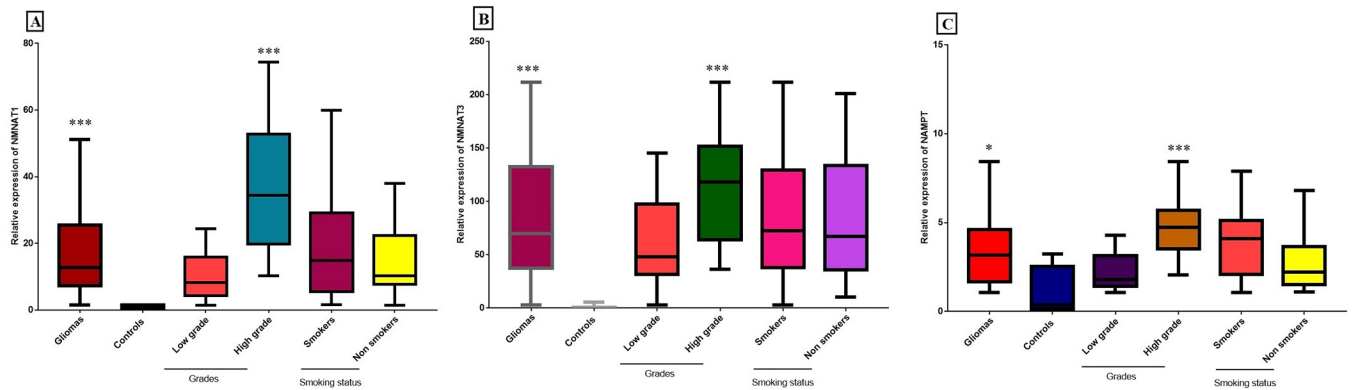
Fig 5. NAD level was measured by relative expression of NAD enzymes. (A) Relative expression level NMNAT1 gene in glioma and controls. Association of relative expression level of NMNAT1 with different grades and smoking status of glioma patients. (B) Relative expression level NMNAT2 gene in glioma and controls. Association of relative expression level of NMNAT2 with different grades and smoking status of glioma patients. (C) Relative expression level NAMPT gene in glioma and controls. Association of relative expression level of NAMPT with different grades and smoking status of glioma patients. $P<0.05^{*}$, $p<0.001^{***}$.

https://doi.org/10.1371/journal.pone.0281840.g005

PARP1 (r = 0.0147, p = 0.129), *GDH* and *OGG1-2α* (r = 0.066, p = 0.568), *SOD2* and *OGG1-2α* (r = 0.3745, p = 0.001***), *OGG1-2α* and *PARP1* (r = 0.009846, p = 0.932), *OGG1-2α* and *HIF1a* (r = 0.01133, p = 0.922), *PARP1* and *HIF1a* (r = 0.0147, p = 0.898), *GDH* and *SOD2* (r = 0.043, p = 0.711), *SOD1* and *OGG1-2α* (r = 0.044, p = 0.699), *SOD2* and *PARP1* (r = -0.2577*, p = 0.02) in glioma patients.

ROC analysis

To assess the diagnostic value of these selected genes, ROC curve analysis was performed as shown in Fig 7. ROC curve showed the area under the curve (AUC) and 95% confidence interval (CI). Analysis showed that calculated area under the curve was 82% for *SIRT3* (AUC = 0.8203; 95%CI = 0.672–0.9685; p<0.0322, Fig 7A), 53% for *SIRT4* (AUC = 0.533; 95% CI = 0.336–0.7307, p<0.0337, Fig 7B), 100% for *SIRT5* (AUC = 1.000; 95%CI = 1.000–1.000; p<0.0001, Fig 7C), 61% for *GDH* (AUC = 0.6127, 95%CI = 0.4720–0.7533, p<0.1582 Fig 7D), 69% for *HIF1a* (AUC = 0.6979; 95%CI = 0.5557–0.8401; p value<0.0155 Fig 7E), 94% for *SOD1*(AUC = 0.9469; 95%CI = 0.9034–0.9903, p<0.0001 Fig 7F), 87% for *SOD2*
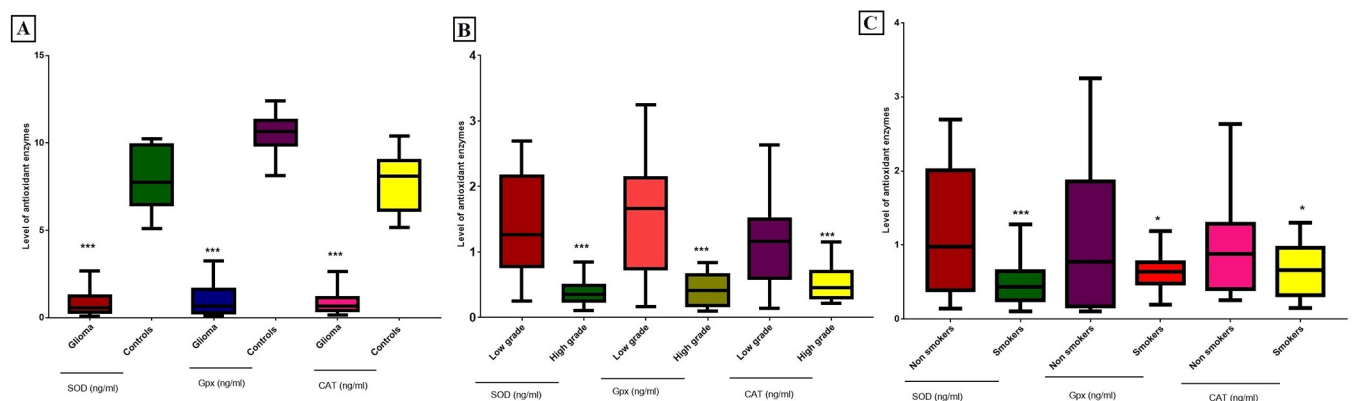

Fig 6. Measurement of oxidative stress by determining the level of antioxidant enzyme SOD, Gpx and CAT in (A) glioma patients vs controls, (B) patients with low grade vs high grade tumor, (C) patients with smoking status and non-smoking status. $P<0.05^{*}$, $p<0.001^{***}$.

https://doi.org/10.1371/journal.pone.0281840.g006

Table 3. Gene-gene correlation of selected genes in glioma patients.

Genes	SIRT4	SIRT5	SOD1	SOD2	HIF1α	PARP1	GDH	OGG1-2α
SIRT3	-0.2213*	-0.0444 ns	0.06661 ns	-0.03706 ns	-0.09681 ns	0.2825*	0.0067 ns	0.2401*
SIRT4		0.0492 ns	-0.03940 ns	0.0241 ns	-0.09176 ns	0.01022 ns	-0.1180 ns	-0.05450 ns
SIRT5			-0.05295 ns	-0.04560 ns	-0.01410 ns	-0.09277 ns	-0.01657 ns	-0.1080 ns
SOD1				-0.1118 ns	-0.417***	-0.212**	0.23**	-0.098 ns
SOD2					0.39***	0.0714 ns	0.02 ns	0.1323 ns
HIF1α						0.2698***	0.0397 ns	0.08 ns
PARP1							-0.08 ns	0.1356 ns
GDH								-0.06 ns

Spearman correlation was performed. ns = non-significant (p>0.05).

*p<0.05

**p<0.01

***p<0.001

https://doi.org/10.1371/journal.pone.0281840.t003

(AUC = 0.8749, 95%CI = 0.8014–0.9484, p<0.0001, Fig 7G), 69% for *OGG1-2α* (AUC = 0.6973, 95%CI = 0.5539–0.8407, p<0.0234, Fig 7H) and 67% for *PARP1* (AUC = 0.6733, 95%CI = 0.5469–0.7996, p<0.0294 Fig 7I) in Glioma patients.

Survival analysis

Kaplan-Meier analysis was performed to check the prognostic value of mitochondrial sirtuins and their associated genes. Survival analysis showed that deregulation of SIRT3 (p<0.0081; Fig 8A), *SIRT4* (p<0.0381; Fig 8B), *SIRT5* (p<0.0151; Fig 8C), *GDH* (p<0.0108; Fig 8F), *OGG1-2α* (p<0.05; Fig 8G), *PARP1* (p<0.0270; Fig 8H) and *HIF1α* (p<0.0053; Fig 8I) was found associated with significant decrease survival of glioma patients as shown in Fig 8. However, nonsignificant difference was observed in case of SOD1 (Fig 8D) and SOD2 (Fig 8E).

Discussion

Gliomas are most common intra-axial brain tumors originated from glial cells [5]. Risk factors for glioma include ionizing radiations, diet, exposure to pesticides, viruses, and genetic factors [35]. Genetic susceptibility also plays an important role in carcinogenesis and different pathways work to maintain genetic mechanisms. These pathways include mitochondrial DNA encoded genes as well as nuclear-encoded mitochondrial proteins [36]. Sirtuin family is among nuclear-encoded mitochondrial proteins, they are family of orthologues of yeast silent information regulator 3 (*SIRT3)*, 4 (*SIRT4)* and 5 (*SIRT5)*. Many studies have been proposed to discuss the role of sirtuins in cancers but still no study has been published to show their role with gliomas. The aim of present study was to elucidate the role of expression deregulations of mitochondrial sirtuin genes and co-expression with their associated proteins (*SOD1*, *SOD2*, *OGG1-2α*, *PARP1*, *GDH and HIF1α*) in glioma. Diagnostic/prognostic value of these genes was also analyzed in the glioma samples to check the role of these genes in glioma diagnosis. Metabolic assay and oxidative stress assay were also performed to assess the nonmetabolic role of selected genes in glioma tumoriogenesis.

SIRT3 is NAD+ dependent mitochondrial deacetylase, it is usually present in nucleus but in stress condition it is transported to mitochondria where it gets trimmed and activates. It regulates many important biological functions like metabolic control, gene expression, aging and many diseases like cancer [37]. Many studies have reported that metabolic programs are linked to tumorigenesis, as *SIRT3* is an important mediator of mitochondrial metabolic pathways so
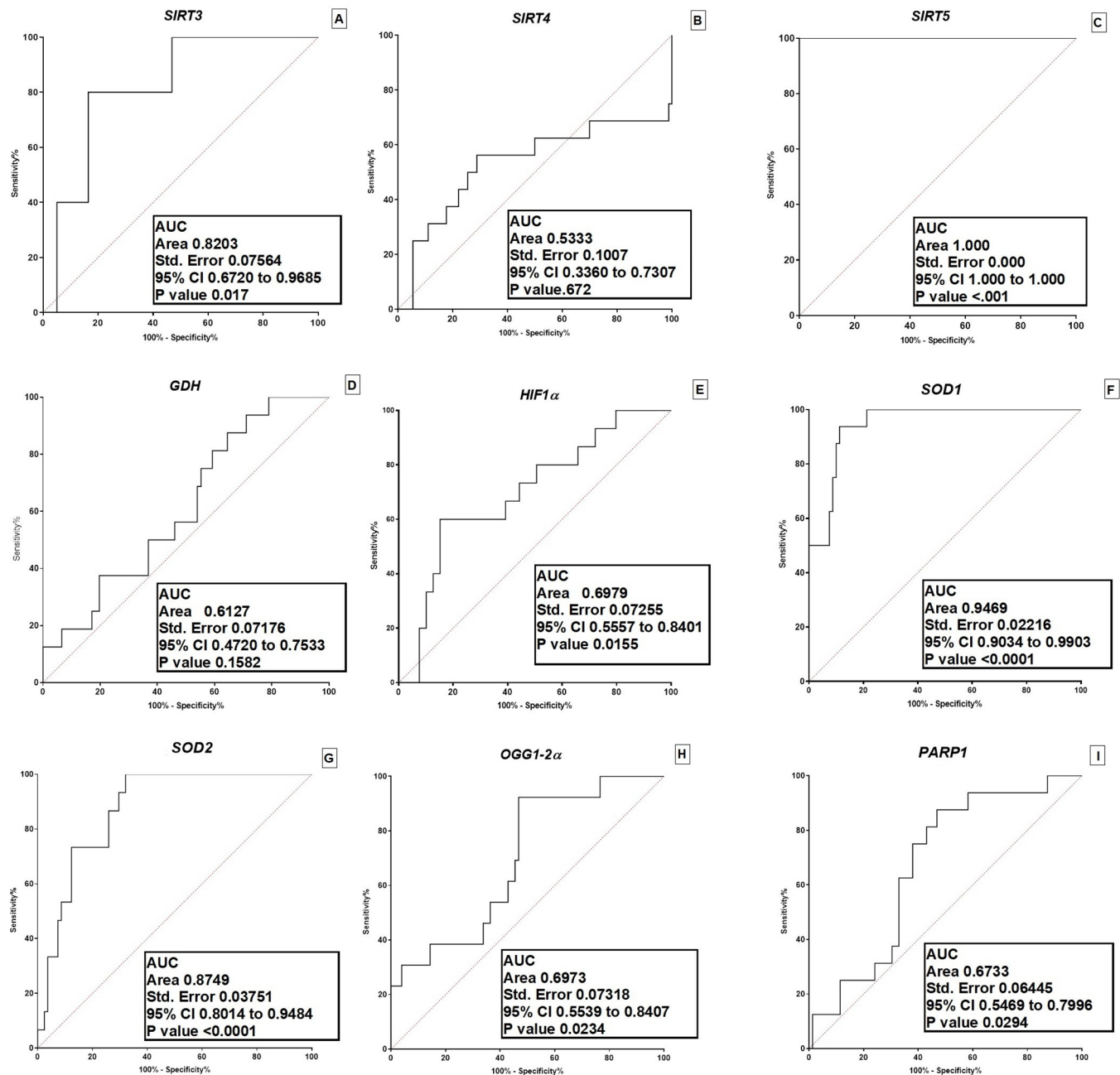
Fig 7. Assessment of diagnostic of selected mitochondrial sirtuins and related genes in glioma patients. (A) Area under curve of SIRT3, (B) area under curve of SIRT4, (C) area under curve of SIRT5, (D) area under curve of GDH, (E) area under curve of HIF1a, (F) area under curve of SOD1, (G) area under curve of SOD1, (H) area under curve of SOD2, (I) area under curve of OGG1-2a and (J) area under curve of PARP1 in glioma patients.

https://doi.org/10.1371/journal.pone.0281840.g007

its overexpression and down-regulation is also linked to the tumor formation or tumor suppression [11]. In present study significant upregulation of *SIRT3* was observed and this upregulation was found linked to the tumor aggressiveness and poor survival. Overexpression of *SIRT3* has been reported in many cancers like in ovarian cancer [38], colon cancer [39] and colorectal cancer [40]. Reason behind the upregulation of SIRT3 gene and increased carcinogenesis needs to be explored. However, our previous study on the involvement of SIRT3 gene
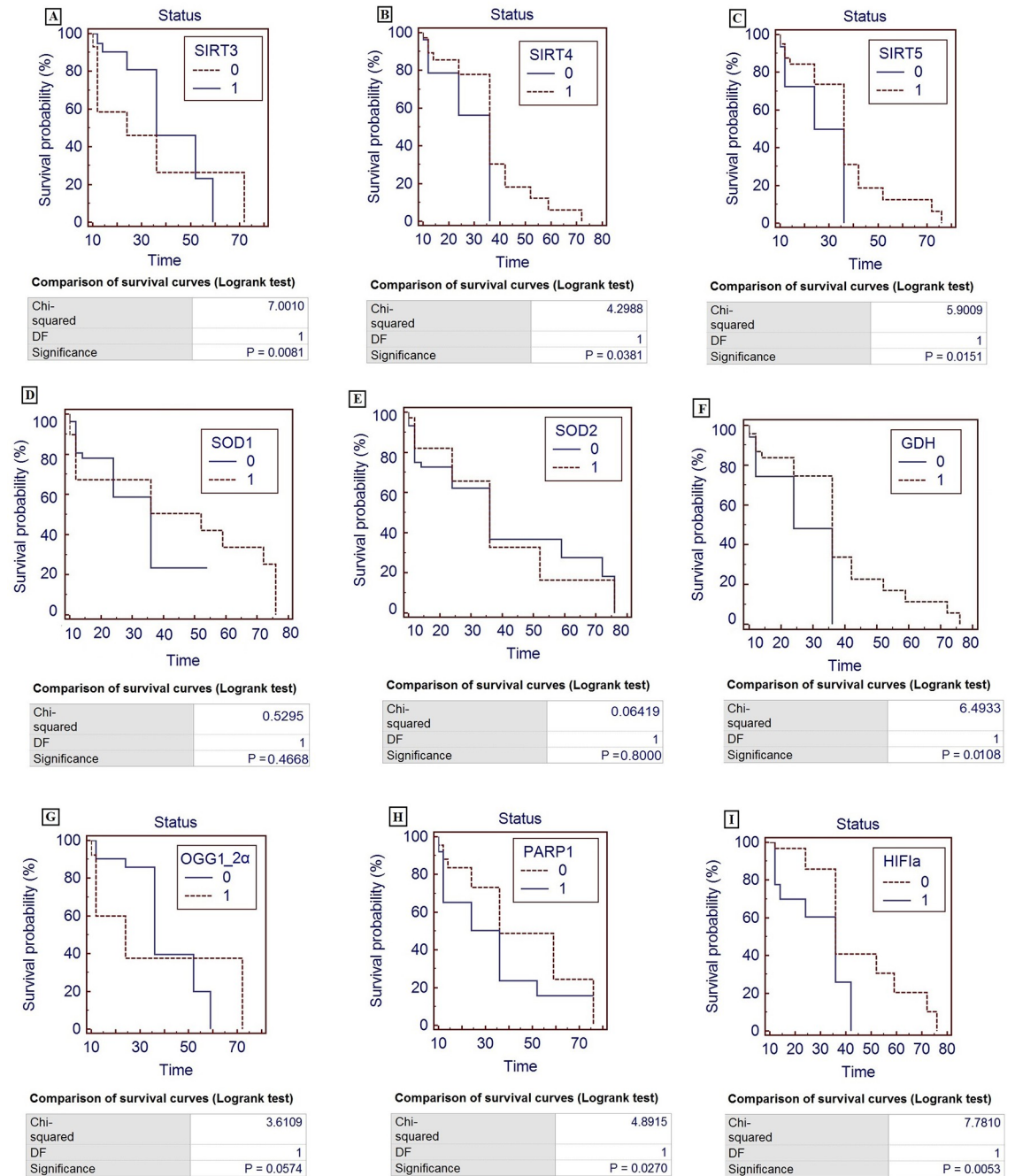
Fig 8. Kaplan meier analysis of selected mitochondrial sirtuins and related genes in glioma patients. (A) Survival curve of SIRT3, (B) survival curve of SIRT4, (C) survival curve of GDH, (D) survival curve of SOD1, (E) survival curve of SOD2, (F) survival curve of GDH, (G) survival curve of OGG1, (H) survival curve of PARP1, (I) survival curve of HIF1a. 0 represents downregulation, 1 represents upregulation. DF is degree of freedom, level of significance p<0.05.

https://doi.org/10.1371/journal.pone.0281840.g008

in carcinogenesis showed that genetic polymorphism in said gene might results in mRNA splicing, epigenetics variations and impaired activity [41]. Previous studies have reported that upregulation of SIRT3 gene may results in reduction in apoptotic power and increased survival capacity of tumor cells, which ultimately leads to increased aggressiveness of tumor [42, 43].

*SIRT4 i*s also found working in mitochondria, it controls the working of glutamate dehy-
drogenase (GDH), which ultimately controls the glutamate and glutamine activity by ADP-
ribosylation. *SIRT4* is found working as suppressor of glutamine under stressful condition, like
oxidative stress and stabilizes the genomic stability [44]. Results from present study showed
the significant downregulation of *SIRT4* in glioma patients compared to healthy controls
depicting its role as a tumor suppressor. Previous studies have also showed the downregulation
of *SIRT4* in gastric [45], thyroid [46] and breast cancer [47]. Mahjabeen & Kayani (2017) have
reported that reason behind the downregulation of SIRT4 gene can be the variation in coding/
noncoding region of said gene, loss of functionality, abnormal metabolic rate, and increased
carcinogenesis [17].

SIRT5 is another member of mitochondrial sirtuins, it is also found working in deacetylase
activity. *SIRT5* binds to glutaminase, which is first enzyme for glutamine metabolism [48]. In
this study SIRT5 is found to be downregulated in glioma patients as compared to the healthy
controls depicting its role as a tumor suppressor. In previous studies *SIRT5* has been found
downregulated in different cancers like liver cancer [48] gastric cancer [49] and hepatocellular
carcinoma [50]. Previous studies have reported the upregulation of SIRT5 gene in breast [51],
ovarian [20] and liver cancer [48]. Reason behind the downregulated and upregulated expres-
sion of SIRT5 gene is not clear yet. However, previous study has reported that high SNPs fre-
quency in coding/noncoding region of SIRT5 gene resulted in deregulation of said genes,
which ultimately results in increased reactive oxygen species, oxidative stress and carcinogene-
sis [52, 53].

SOD1 is mitochondrial protein and act as an antioxidant enzyme. It is involved in forma-
tion of hydrogen peroxide to superoxide. SOD1 is a cytosolic protein but is also localized in
inter-membrane spaces of mitochondria [54, 55]. In present study SOD1 expression was
found downregulated in gliomas compared to healthy control tissues which are in line with
the previously reported findings. In previous studies, SOD1 has found to be overexpressed in
numerous cancers like NSCLC [22], leukemia [56] and breast cancer [57], the overexpression
is linked to poor survival of cancer cell [22]. Although *SOD1* is highly expressed and act as
oncogene in many cancers but in pancreatic cancer cells *SOD1* has differentially expressed. It
indicated that it is tissue-specific and play different and opposite roles in different types of can-
cers. High *SOD1* expression promotes tumor cell proliferation, it can be, by reduced antioxi-
dant protein activity leading to increased intracellular H2O2. *SOD1* inhibition increases
reactive oxygen species levels and results in increased DNA double strand breaks and carcino-
genesis [22].

SOD2 is a key antioxidant, and it is localized in mitochondria and is found to act as poten-
tial tumor suppressor gene in carcinogenesis [41]. SOD2 has also showed differential expres-
sion in different cancers such as cervical [58], lung [59], brain [60], colon [61], breast [62],
gastric [63] and salivary cancer [64]. This overexpression has been found linked to metastasis,
poor prognosis and lower survival. In another study SOD2 expression has been found to be
downregulated in cancerous cells as compared to control cells [24]. In present study SOD2 has
found to be downregulated, one of previous study has demonstrating the similar findings as it
has been reported that SOD2 showed downregulated expression in breast, esophageal and pan-
creatic cancers [65].

OGG1-2α is DNA repair gene and low expression of DNA repair gene results in reduced
DNA repair activity resulting in more oxidative imbalance [66]. In previous studies *OGG1-2α*
has been found to be down regulated in carcinogenic cells compared to controls suggesting the
systems of excision of bases operate without any disability [67]. It has been found downregu-
lated in colorectal cancer [26] and gastric cancer, which might be due to genetic heterogeneity
in *OGG1-2α* gene because of environmental, genetic, and epigenetic factors [68].

Overexpression of *OGG1-2α* has been found in one study in lung cancer which is because of the polymorphism Ser326Cys. It has been found that variation of Cys326/Cys326 genotype has high risk of lung cancer than wild type allele carriers, statistically significant interaction was found between genotype and mRNA [69]. In a recent leukemia study, *OGG12* has been found downregulated as it is key factor of cancer cells that DNA repair systems are deregulated hence decreased expression of the gene [70]. In present study, expression of *OGG12* is found to be downregulated in glioma tissues compared to controls thus, strongly in accordance with previously published results [71].

PARP1 senses the nicks in single stranded and double stranded DNA breaks and seals them caused by oxidative stress. It is found to be hyperactive in oxidative stress and this hyperactivation also leads to cell death [72]. The stressful conditions like inflammation excitotoxicity, ischemia, and oxidative stress cause over activation of *PARP1* [73]. In previous studies it has been found to be overexpressed in various tumors like breast, ovary, skin, brain and lungs and this upregulation is linked to the enhanced anti-apoptotic property of tumors [74]. It has also found overexpressed in NSCLC [75], prostate [76], colorectal cancer [77] and gliomas [74]. Overexpression of *PARP1* is linked to poor survival overall in cancer [74]. In current study the expression of *PARP1* was also found over expressed. *PARP1* is found to be linked with the mechanisms relating to proapoptotic mechanisms [76] and overexpression might be due to defective cleavage of *PARP1* and reduced apoptosis hence long survival of cancer cells [33].

HIF1α is main regulator of hypoxia, cell cycle, metastasis, angiogenesis, metabolism and proliferation [22, 78]. Over expression of *HIF1α* has been observed in different cancers such as gastric [79] pancreatic cancer [80], NSCLC [81], thyroid cancer [82] and leukemia [83]. In current study *HIF1α* was found to be upregulated in glioma as compared to the controls supporting the previous results. Chen *et al.*, (2020) has reported that upregulation of *HIF1α* was found to be associated with activation of other oncogenic pathways like anti-tumor immunosuppression, cell cycle, EMT etc. This association has showed tumor promoter role of HIF1α [22]. HIF1α is stabilized with high ROS levels and provides advantage to tumor cells in division [78].

GDH plays important role in glutamine metabolism and its role in tumor cells is of great interest. GDH plays key role in glutathione which is important for maintaining redox balance in cell [84]. In this study GDH is found to be downregulated in glioma compared to healthy control samples. Deregulation of GDH has been observed in several cancers like thyroid cancer [85], head and neck cancer [86], breast cancer [87], lung cancer [21] and gliomas [88]. It has been found downregulated in lung cancer patients [89]. Expression level of mitochondrial sirtuins was correlated with selected related genes and significant correlation was observed between mitochondrial sirtuins and selected genes. This correlation results showed the combine involvement of selected gene in increased risk of glioma. Diagnostic and prognostic values of selected genes were also measured in present study. ROC curve analysis was performed and >80 percent diagnostic sensitivity was measured in case of SIRT3 and SIRT5 genes in glioma patients. In case of prognostic values of selected genes Kaplan Meier analysis was performed and significant deregulated expression of mitochondrial sirtuins was found linked with the decreased survival rate in glioma patients. Tan et al., (2020) has also reported that deregulation of mitochondrial sirtuins has found associated with decrease survival and increased hazard ratio of lower grade glioma [90]. Previous study has reported the downregulation of SIRT5 gene in glioblastoma and this deregulation was found associated with poor prognosis [91]. Prognostic values of mitochondrial sirtuins have been reported in different cancers such as breast cancer, colon cancer and non- small cell lung carcinoma [92]. Fu et al., (2020) has reported the prognostic value of mitochondrial situins in prostate cancer [93].

In present study DNA damage was measured by single cell gel electrophoresis and significant high number of comets were observed in glioma patients compared to the controls.

Along with this, some other parameters like %DNA in tail, olive tail moment and tail length were also analyzed, and they were found significantly high in glioma samples compared to healthy samples. DNA damage is any physical or chemical change in DNA in cells, in its result interpretation and transmission of genetic information is affected. The damage can be done by multiple factors like chemicals, radiations free radicles and topological changes which ultimately all result in different forms of damage. ROS is well recognized DNA damage mediator [94]. In present study, oxidative stress was also assessed by measuring the antioxidant level and significant increased oxidative stress was observed. ROS also induces DNA damage by oxidizing nucleoside bases (8-oxo guanine) which can lead to G-A and G-T transversions if unrepaired. ROS accumulation also results in mitochondrial DNA lesions, strand breaks and degradation of mitochondrial DNA [95].

Metabolic assays like ATP, NAD+ and glutathione level determination were also performed. In gliomas metabolic pathways like glycolysis, lipid and amino acid metabolism are found to be abnormally regulated. As ATP is the core of these pathways it has inevitably significant impact on glioma behaviors [96]. ATP is found to be at low levels in extracellular fluids in healthy tissues. While in case of some diseases including cancer, ATP level is increased [97], like in gastric cancer [98]. In our study level of ATP was also found high compared to the healthy controls.

Nicotinamide adenine dinucleotide (NAD) is a co-enzyme that actively involved in regulation of metabolic pathway. These pathways are serine biosynthesis, glycolysis and tricarboxylic acid (TCA) cycle [99, 100]. As NAD is restored so frequently and act as fuel for tumor cell, promote the uncontrolled cell division, proliferation, and survival. Elevated levels of NAD enhance glycolysis by the GAPDH and LDH, as they require NAD as coenzyme. NAD also serves as substrate for sirtuins and PARP hence mediate deacetylation and poly-ADP-ribosylation [101]. Intracellular NAD in mammals is controlled by two enzymes nicotinamide phosphoribosyltransferase (NAMPT) and nicotineamide mononucleotide adenyltransferase (Nmnat) [100]. In mammals Nmnat have three isozymes and they have different subcellular locations. NMNAT1 in nucleus, Nmnat2 in golgi apparatus, NMNAT3 in mitochondria and NAMPT is located in cytoplasm [102, 103]. In mitochondria, NAD utilization is in TCA cycle, oxidative phosphorylation, and Fatty acid oxidation [104]. In our study NAMPT, NMNAT1 and NMNAT3 were found significantly upregulated in glioma samples compared to the controls. Song et al., (2013) has reported that NMNAT1 is found located on chromosomal region which gets deleted frequently in cancers such as in lung cancer, ultimately resulting in advantage to tumor development [105]. Limited number of studies have been reported for NMNAT3. Anderson et al., (2017) has reported increased expression of NMNAT3, NAD level and enhanced energy level in mouse model [106]. NAMPT has been reported upregulated in different cancers like, colorectal cancer [107] prostate, breast and gastric cancers and this dysregulation is linked to promote tumor glycolysis, metastasis, invasion, proliferation, survival, and chemotherapy resistance [108].

Conclusion

The present study demonstrated the expression variation of selected genes in glioma patients. The deregulation of these genes along with other metabolism deregulation resulted in increased oxidative stress hence resulting in unrepairable DNA damage and reduced survival in glioma patients. This study also provides potent insights into use of mitochondrial sirtuins and related genes as diagnostic and prognostic biomarkers. Several limitations are needed to be considered in present study such as study should incorporate oxygen consumption rate of glioma patients using seahorse analysis for better understanding of role of mitochondrial

abnormalities in glioma patients. Our study size is small, further validation studies with large sample size should be done to illuminate the mechanistic role of selected gene in cancerogenesis of different region including glioma.

Acknowledgments

Author Contributions

Conceptualization: Ishrat Mahjabeen.

Data curation: Maria Fazal Ul Haq, Muhammad Zahid Hussain, Zertashia Akram, Sumaira Fida Abbasi, Mahmood Akhtar Kayani.

Formal analysis: Maria Fazal Ul Haq, Rabia Shafique.

Investigation: Maria Fazal Ul Haq, Muhammad Zahid Hussain, Nadia Saeed, Rabia Shafique, Sumaira Fida Abbasi.

Methodology: Maria Fazal Ul Haq, Zertashia Akram, Nadia Saeed, Rabia Shafique, Sumaira Fida Abbasi.

Project administration: Muhammad Zahid Hussain, Sumaira Fida Abbasi.

Software: Maria Fazal Ul Haq, Muhammad Zahid Hussain, Zertashia Akram, Nadia Saeed, Rabia Shafique, Mahmood Akhtar Kayani.

Supervision: Mahmood Akhtar Kayani.

Validation: Ishrat Mahjabeen, Mahmood Akhtar Kayani.

Visualization: Ishrat Mahjabeen, Mahmood Akhtar Kayani.

Writing – original draft: Ishrat Mahjabeen, Mahmood Akhtar Kayani.

Writing – review & editing: Ishrat Mahjabeen, Mahmood Akhtar Kayani.

References

1. Hill JR, Kuriyama N, Kuriyama H, Israel MA. Molecular genetics of brain tumors. Archives of neurology. 1999; 56(4):439–41. https://doi.org/10.1001/archneur.56.4.439 PMID: 10199332.

2. Afshar P, Mohammadi A, Plataniotis KN. Brain tumor type classification via capsule networks. arXiv preprint. arXiv preprint arXiv:1802.10200. 2018.

3. International Agency for Research on Cancer. Available online: https://gco.iarc.fr/

4. Cheng Y, Dai C, Zhang J. SIRT3-SOD2-ROS pathway is involved in linalool-induced glioma cell apoptotic death. Acta Biochimica Polonica. 2017; 64(2):343–50. https://doi.org/10.18388/abp.2016_1438 PMID: 28567457.

5. Yusoff AA, Khair SZ, Abd Radzak SM, Idris Z, Lee HC. Prevalence of mitochondrial DNA common deletion in patients with gliomas and meningiomas: a first report from a Malaysian study group. Journal of the Chinese Medical Association. 2020; 83(9):838. https://doi.org/10.1097/JCMA.0000000000000401 PMID: 32732530.

6. Pagano C, Navarra G, Coppola L, Avilia G, Pastorino O, Monica RD, et al. N6-isopentenyladenosine induces cell death through necroptosis in human glioblastoma cells. Cell death discovery, (2022); 8:173: https://doi.org/10.1038/s41420-022-00974-x PMID: 35393392

7. Sainz RM, Lombo F, Mayo JC. Radical decisions in cancer: redox control of cell growth and death. Cancers. 2012; 4(2):442–74. https://doi.org/10.3390/cancers4020442 PMID: 24213319.

8.   Chen Y, Qin C, Huang J, Tang X, Liu C, Huang K, et al. The role of astrocytes in oxidative stress of central nervous system: A mixed blessing. Cell proliferation. 2020; 53(3):e12781. https://doi.org/10.1111/cpr.12781 PMID: 32035016.

9.   Yusoff AA. Role of mitochondrial DNA mutations in brain tumors: A mini-review. Journal of cancer research and therapeutics. 2015; 11(3):535. https://doi.org/10.4103/0973-1482.161925 PMID: 26458578.

10. Chu Q, Gu X, Zheng Q, Wang J, Zhu H. Mitochondrial Mechanisms of Apoptosis and Necroptosis in Liver Diseases. Analytical cellular pathology, (2021); 9: 8900122: https://doi.org/10.1155/2021/8900122 PMID: 34804779

11. Kim HS, Patel K, Muldoon-Jacobs K, Bisht KS, Aykin-Burns N, Pennington JD, et al. SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. Cancer cell. 2010; 17(1):41–52. https://doi.org/10.1016/j.ccr.2009.11.023 PMID: 20129246.

12. Jeong S. M., Xiao C., Finley L. W., Lahusen T., Souza A. L., Pierce K., et al. (2013). SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glutamine metabolism. *Cancer cell*, 23(4), 450–463. https://doi.org/10.1016/j.ccr.2013.02.024 PMID: 23562301

13. Chen Y. L., Fu L. L., Wen X., Wang X. Y., Liu J., Cheng Y., et al. (2014). Sirtuin-3 (SIRT3), a therapeutic target with oncogenic and tumor-suppressive function in cancer. *Cell death & disease*, 5(2), e1047. https://doi.org/10.1038/cddis.2014.14 PMID: 24503539

14. Torrens-Mas M, Oliver J, Roca P, Sastre-Serra J. SIRT3: oncogene and tumor suppressor in cancer. Cancers. 2017; 9(7):90. https://doi.org/10.3390/cancers9070090 PMID: 28704962.

15. Vassilopoulos A., Fritz K. S., Petersen D. R., & Gius D. (2011). The human sirtuin family: evolutionary divergences and functions. *Human genomics*, 5(5), 485. https://doi.org/10.1186/1479-7364-5-5-485 PMID: 21807603

16. Ul Haq MF, Kayani MA, Arshad T, Hadi Anwar RA, Saeed N, Shafique R, et al. Genetic interactions of mitochondrial sirtuins in brain tumorigenesis. Future Oncology. 2022 Feb; 18(5):597–611. https://doi.org/10.2217/fon-2021-0264 PMID: 35034477

17. Hu Y, Lin J, Lin Y, Chen X, Zhu G, Huang G. Overexpression of SIRT4 inhibits the proliferation of gastric cancer cells through cell cycle arrest. Oncology Letters. 2019; 17(2):2171–6. https://doi.org/10.3892/ol.2018.9877 PMID: 30745932.

18. Mahjabeen I, Kayani MA. Loss of mitochondrial tumor suppressor genes expression is associated with unfavorable clinical outcome in head and neck squamous cell carcinoma: Data from retrospective study. PLoS One. 2016; 11(1):e0146948. https://doi.org/10.1371/journal.pone.0146948 PMID: 26785117.

19. Bringman-Rodenbarger LR, Guo AH, Lyssiotis CA, Lombard DB. Emerging roles for SIRT5 in metabolism and cancer. Antioxidants & redox signaling. 2018; 28(8):677–90. https://doi.org/10.1089/ars.2017.7264 PMID: 28707979.

20. Yang W, van de Ven RA, Haigis MC. Mitochondrial sirtuins: coordinating stress responses through regulation of mitochondrial enzyme networks. In Introductory Review on Sirtuins in Biology, Aging, and Disease. 2018(pp. 95–115). Academic Press.

21. Kumar S, Lombard DB. Mitochondrial sirtuins and their relationships with metabolic disease and cancer. Antioxidants & redox signaling. 2015; 22(12):1060–77. https://doi.org/10.1089/ars.2014.6213 PMID: 25545135.

22. Chen B, Li L, Li M, Wang X. HIF1A expression correlates with increased tumor immune and stromal signatures and aggressive phenotypes in human cancers. Cellular Oncology. 2020; 43(5):877–88. https://doi.org/10.1007/s13402-020-00534-4 PMID: 32488852.

23. Liu S, Li B, Xu J, Hu S, Zhan N, Wang H, et al. SOD1 promotes cell proliferation and metastasis in non-small cell lung cancer via an miR-409-3p/SOD1/SETDB1 epigenetic regulatory feedforward loop. Frontiers in cell and developmental biology. 2020; 8:213. https://doi.org/10.3389/fcell.2020.00213 PMID: 32391354.

24. Lisse TS. Vitamin D regulation of a SOD1-to-SOD2 antioxidative switch to prevent bone cancer. Applied Sciences. 2020; 10(7):2554.

25. Zhang LF, Xu K, Tang BW, Zhang W, Yuan W, Yue C, et al. Association between SOD2 V16A variant and urological cancer risk. Aging (Albany NY). 2020; 12(1):825. https://doi.org/10.18632/aging.102658 PMID: 31929112.

26. Vlahopoulos S, Adamaki M, Khoury N, Zoumpourlis V, Boldogh I. Roles of DNA repair enzyme OGG1 in innate immunity and its significance for lung cancer. Pharmacology & therapeutics. 2019; 194:59–72. https://doi.org/10.1016/j.pharmthera.2018.09.004 PMID: 30240635.

27. Santos JC, Funck A, Silva-Fernandes IJ, Rabenhorst SH, Martinez CA, Ribeiro ML. Effect of APE1 T2197G (Asp148Glu) polymorphism on APE1, XRCC1, PARP1 and OGG1 expression in patients with colorectal cancer. International journal of molecular sciences. 2014; 15(10):17333–43. https://doi.org/10.3390/ijms151017333 PMID: 25268610.

28. Khan AU, Mahjabeen I, Malik MA, Hussain MZ, Khan S, Kayani MA. Modulation of brain tumor risk by genetic SNPs in PARP1gene: Hospital based case control study. Plos one. 2019; 14(10):e0223882. https://doi.org/10.1371/journal.pone.0223882 PMID: 31609976.

29. Schlicker C, Gertz M, Papatheodorou P, Kachholz B, Becker CF, Steegborn C. Substrates and regulation mechanisms for the human mito- chondrial sirtuins Sirt3 and Sirt5. J Mol Biol 2008; 382: 790–801. https://doi.org/10.1016/j.jmb.2008.07.048 PMID: 18680753

30. Singh C.; Singh C.K.; Chhabra G.; Nihal M.; Iczkowski K.A.; Ahmad N. Abstract 539: Pro-proliferative function of the histone deacetylase SIRT3 in prostate cancer. Cancer Res. 2018, 78, 539.

31. Klemm F, Maas RR, Bowman RL, Kornete M, Soukup K, Nassiri S, et al. Interrogation of the microenvironmental landscape in brain tumors reveals disease-specific alterations of immune cells. Cell. 2020; 181(7):1643–60. https://doi.org/10.1016/j.cell.2020.05.007 PMID: 32470396

32. Griesinger AM, Birks DK, Donson AM, Amani V, Hoffman LM, Waziri A, et al, Handler MH, Foreman NK. Characterization of distinct immunophenotypes across pediatric brain tumor types. The Journal of Immunology. 2013; 191(9):4880–8.

33. Afzal H, Yousaf S, Rahman F, Ahmed MW, Akram Z, Kayani MA, Mahjabeen I. PARP1: A potential biomarker for gastric cancer. Pathology-Research and Practice. 2019; 215(8):152472. https://doi.org/10.1016/j.prp.2019.152472 PMID: 31174925.

34. Akram Z, Riaz S, Kayani MA, Jahan S, Ahmad MW, Ullah MA, et al. Lead induces DNA damage and alteration of ALAD and antioxidant genes mRNA expression in construction site workers. Archives of Environmental & Occupational Health. 2019; 74(4):171–8. https://doi.org/10.1080/19338244.2018.1428523 PMID: 29336731.

35. Crump C, Sundquist J, Sieh W, Winkleby MA, Sundquist K. Perinatal and Familial Risk Factors for Brain Tumors in Childhood through Young Adulthood Risk Factors for Brain Tumors. Cancer research. 2015; 75(3):576–83. https://doi.org/10.1158/0008-5472.CAN-14-2285 PMID: 25511376.

36. Chinnery PF, Hudson G. Mitochondrial genetics. British medical bulletin. 2013; 106(1):135–59. https://doi.org/10.1093/bmb/ldt017 PMID: 23704099.

37. Alhazzazi TY, Kamarajan P, Joo N, Huang JY, Verdin E, D'Silva NJ, et al. Sirtuin-3 (SIRT3), a novel potential therapeutic target for oral cancer. Cancer. 2011; 117(8):1670–8. https://doi.org/10.1002/cncr.25676 PMID: 21472714.

38. Kim YS, Gupta Vallur P, Jones VM, Worley BL, Shimko S, Shin DH, et al. Context-dependent activation of SIRT3 is necessary for anchorage-independent survival and metastasis of ovarian cancer cells. Oncogene. 2020; 39(8):1619–33. https://doi.org/10.1038/s41388-019-1097-7 PMID: 31723239.

39. Liu Z, Li L, Xue B. Effect of ganoderic acid D on colon cancer Warburg effect: Role of SIRT3/cyclophilin D. European journal of pharmacology. 2018; 824:72–7. https://doi.org/10.1016/j.ejphar.2018.01.026 PMID: 29374515.

40. Wang Y, Sun X, Ji K, Du L, Xu C, He N, et al. Sirt3-mediated mitochondrial fission regulates the colorectal cancer stress response by modulating the Akt/PTEN signalling pathway. Biomedicine & Pharmacotherapy. 2018; 105:1172–82. https://doi.org/10.1016/j.biopha.2018.06.071 PMID: 30021354.

41. Ahmed MW, Mahjabeen I, Gul S, Khursheed A, Mehmood A, Kayani MA. Relationship of single nucleotide polymorphisms and haplotype interaction of mitochondrial unfolded protein response pathway genes with head and neck cancer. Future Oncology. 2019; 15(33):3819–29. https://doi.org/10.2217/fon-2019-0365 PMID: 31651195.

42. Sundaresan NR, Samant SA, Pillai VB, Rajamohan SB, Gupta MP. SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70. Molecular and cellular biology. 2008; 28(20):6384–401. https://doi.org/10.1128/MCB.00426-08 PMID: 18710944.

43. Chen Y, Fu LL, Wen X, Wang XY, Liu J, Cheng Y, et al. Sirtuin-3 (SIRT3), a therapeutic target with oncogenic and tumor-suppressive function in cancer. Cell Death and Disease. 2014; 5(2): e1047. https://doi.org/10.1038/cddis.2014.14 PMID: 24503539.

44. Yu W, Denu RA, Krautkramer KA, Grindle KM, Yang DT, Asimakopoulos F, et al. Loss of SIRT3 provides growth advantage for B cell malignancies. Journal of Biological Chemistry. 2016; 291(7):3268–79. https://doi.org/10.1074/jbc.M115.702076 PMID: 26631723.

45. Sun H, Huang D, Liu G, Jian F, Zhu J, Zhang L. SIRT4 acts as a tumor suppressor in gastric cancer by inhibiting cell proliferation, migration, and invasion. OncoTargets and therapy. 2018; 11:3959. https://doi.org/10.2147/OTT.S156143 PMID: 30022839.

46. Chen Z, Lin J, Feng S, Chen X, Huang H, Wang C, et al. SIRT4 inhibits the proliferation, migration, and invasion abilities of thyroid cancer cells by inhibiting glutamine metabolism. OncoTargets and therapy. 2019; 12:2397. https://doi.org/10.2147/OTT.S189536 PMID: 30992675.

47. Du L, Liu X, Ren Y, Li J, Li P, Jiao Q, et al. Loss of SIRT4 promotes the self-renewal of Breast Cancer Stem Cells. Theranostics. 2020; 10(21):9458. https://doi.org/10.7150/thno.44688 PMID: 32863939.

48. Chen XF, Tian MX, Sun RQ, Zhang ML, Zhou LS, Jin L, et al. SIRT 5 inhibits peroxisomal ACOX 1 to prevent oxidative damage and is downregulated in liver cancer. EMBO reports. 2018; 19(5):e45124. https://doi.org/10.15252/embr.201745124 PMID: 29491006.

49. Lu X, Yang P, Zhao X, Jiang M, Hu S, Ouyang Y, et al. OGDH mediates the inhibition of SIRT5 on cell proliferation and migration of gastric cancer. Experimental Cell Research. 2019; 382(2):111483. https://doi.org/10.1016/j.yexcr.2019.06.028 PMID: 31247190.

50. Guo D, Song X, Guo T, Gu S, Chang X, Su T, et al. Vimentin acetylation is involved in SIRT5-mediated hepatocellular carcinoma migration. American journal of cancer research. 2018; 8(12):2453. PMID: 30662803.

51. Wang Y, Guo Y, Gao J, Yuan X. Tumor-suppressive function of SIRT4 in neuroblastoma through mitochondrial damage. Cancer Management and Research. 2018; 10:5591. https://doi.org/10.2147/CMAR.S172509 PMID: 30519106.

52. Csibi A, Fendt SM, Li C, Poulogiannis G, Choo AY, Chapski DJ, et al. The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4 (vol 153, pg 840, 2013). Cell. 2021; 184(8):2256–. https://doi.org/10.1016/j.cell.2021.03.059 PMID: 33861966.

53. Polletta L, Vernucci E, Carnevale I, Arcangeli T, Rotili D, Palmerio S, et al. SIRT5 regulation of ammonia-induced autophagy and mitophagy. Autophagy. 2015; 11(2):253–70. https://doi.org/10.1080/15548627.2015.1009778 PMID: 25700560.

54. Gomez M, Germain D. Cross talk between SOD1 and the mitochondrial UPR in cancer and neurodegeneration. Molecular and Cellular Neuroscience. 2019; 98:12–8. https://doi.org/10.1016/j.mcn.2019.04.003 PMID: 31028834.

55. Papa L, Manfredi G, Germain D. SOD1, an unexpected novel target for cancer therapy. Genes & cancer. 2014; 5(1–2):15. https://doi.org/10.18632/genesandcancer.4 PMID: 24955214.

56. Davis SM, Collier LA, Leonardo CC, Seifert HA, Ajmo CT, Pennypacker KR. Leukemia inhibitory factor protects neurons from ischemic damage via upregulation of superoxide dismutase 3. Molecular neurobiology. 2017; 54(1):608–22. https://doi.org/10.1007/s12035-015-9587-2 PMID: 26746670

57. Li K, Liu T, Chen J, Ni H, Li W. Survivin in breast cancer–derived exosomes activates fibroblasts by up-regulating SOD1, whose feedback promotes cancer proliferation and metastasis. Journal of Biological Chemistry. 2020; 295(40):13737–52. https://doi.org/10.1074/jbc.RA120.013805 PMID: 32709750.

58. Rabelo-Santos SH, Termini L, Boccardo E, Derchain S, Longatto-Filho A, Andreoli MA, et al. Strong SOD2 expression and HPV-16/18 positivity are independent events in cervical cancer. Oncotarget. 2018; 9(31):21630. https://doi.org/10.18632/oncotarget.24850 PMID: 29774090

59. Ma CS, Lv QM, Zhang KR, Tang YB, Zhang YF, Shen Y, et al. NRF2-GPX4/SOD2 axis imparts resistance to EGFR-tyrosine kinase inhibitors in non-small-cell lung cancer cells. Acta Pharmacologica Sinica. 202; 42(4):613–23. https://doi.org/10.1038/s41401-020-0443-1 PMID: 32704041.

60. Chang KY, Chien CH, Chu JM, Chung PH, Liu MS, Chuang JY. Mitochondrial SOD2 is the mainstay to protect the stemness-featured glioblastoma cells against drug-induced reactive oxygen stress. Cancer Research. 2018;78(13_Supplement):4887–.

61. Potočnjak I, Šimić L, Gobin I, Vukelić I, Domitrović R. Antitumor activity of luteolin in human colon cancer SW620 cells is mediated by the ERK/FOXO3a signaling pathway. Toxicology in Vitro. 2020; 66:104852. https://doi.org/10.1016/j.tiv.2020.104852 PMID: 32268164.

62. He C, Danes JM, Hart PC, Zhu Y, Huang Y, de Abreu AL, et al. SOD2 acetylation on lysine 68 promotes stem cell reprogramming in breast cancer. Proceedings of the National Academy of Sciences. 2019; 116(47):23534–41. https://doi.org/10.1073/pnas.1902308116 PMID: 31591207.

63. Liu YD, Yu L, Ying L, Balic J, Gao H, Deng NT, et al. Toll-like receptor 2 regulates metabolic reprogramming in gastric cancer via superoxide dismutase 2. International journal of cancer. 2019; 144 (12):3056–69. https://doi.org/10.1002/ijc.32060 PMID: 30536754

64. Jung CH, Kim EM, Song JY, Park JK, Um HD. Mitochondrial superoxide dismutase 2 mediates γ-irradiation-induced cancer cell invasion. Experimental & Molecular Medicine. 2019; 51(2):1–0. https://doi.org/10.1038/s12276-019-0207-5 PMID: 30755594

65. Wang R, Yin C, Li XX, Yang XZ, Yang Y, Zhang MY, et al. Reduced SOD2 expression is associated with mortality of hepatocellular carcinoma patients in a mutant p53-dependent manner. Aging (Albany NY). 2016; 8(6):1184. https://doi.org/10.18632/aging.100967 PMID: 27221200

66. Shinmura K, Yokota J. The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis. Antioxidants and Redox Signaling. 2001; 3(4):597–609. https://doi.org/10.1089/15230860152542952 PMID: 11554447

67. Nascimento EF, Ribeiro ML, Magro DO, Carvalho J, Kanno DT, Martinez CA, et al. Tissue expresion of the genes mutyh and ogg1 in patients with sporadic colorectal cancer. ABCD. Arquivos Brasileiros de Cirurgia Digestiva (São Paulo). 2017 Apr; 30:98–102. https://doi.org/10.1590/0102-6720201700020005 PMID: 29257843.

68. Yousaf S, Khan AU, Akram Z, Kayani MA, Nadeem I, Begum B, et al. Expression deregulation of DNA repair pathway genes in gastric cancer. Cancer Genetics. 2019; 237:39–50. https://doi.org/10.1016/j.cancergen.2019.06.002 PMID: 31447064

69. Hatt L, Loft S, Risom L, Møller P, Sørensen M, Raaschou-Nielsen O, et al. OGG1 expression and OGG1 Ser326Cys polymorphism and risk of lung cancer in a prospective study. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2008; 639(1–2):45–54. https://doi.org/10.1016/j.mrfmmm.2007.11.002 PMID: 18155253.

70. Zoraiz K, Attique M, Shahbaz S, Ahmed MW, Kayani MA, Mahjabeen I. Deregulation of mitochondrial sirtuins and OGG1-2a acts as a prognostic and diagnostic biomarker in leukemia. Future Oncology. 2021; 17(27):3561–77. https://doi.org/10.2217/fon-2020-1155 PMID: 34189942.

71. Brain Tumor Basics. Available online: https://www.thebraintumourcharity.org/

72. Hou D, Liu Z, Xu X, Liu Q, Zhang X, Kong B, et al. Increased oxidative stress mediates the antitumor effect of PARP inhibition in ovarian cancer. Redox Biology. 17: 99–111. https://doi.org/10.1016/j.redox.2018.03.016 PMID: 29684820.

73. Ansari A, Rahman MS, Saha SK, Saikot FK, Deep A, Kim KH. Function of the SIRT 3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. Aging cell. 2017; 16(1):4–16. https://doi.org/10.1111/acel.12538 PMID: 27686535

74. Murnyák B, Kouhsari MC, Hershkovitch R, Kálmán B, Marko-Varga G, Klekner Á, et al. PARP1 expression and its correlation with survival is tumour molecular subtype dependent in glioblastoma. Oncotarget. 2017; 8(28):46348. https://doi.org/10.18632/oncotarget.18013 PMID: 28654422.

75. Chen K, Li Y, Xu H, Zhang C, Li Z, Wang W, et al. An analysis of the gene interaction networks identifying the role of PARP1 in metastasis of non-small cell lung cancer. Oncotarget. 2017; 8(50):87263. https://doi.org/10.18632/oncotarget.20256 PMID: 29152079

76. Salemi M, Galia A, Fraggetta F, La Corte C, Pepe P, La Vignera S, et al. Poly (ADP-ribose) polymerase 1 protein expression in normal and neoplastic prostatic tissue. European journal of histochemistry: EJH. 2013; 57(2). https://doi.org/10.4081/ejh.2013.e13 PMID: 23807292.

77. Dziaman T, Ludwiczak H, Ciesla JM, Banaszkiewicz Z, Winczura A, Chmielarczyk M, et al. PARP-1 expression is increased in colon adenoma and carcinoma and correlates with OGG1. PloS one. 2014; 9(12):e115558. https://doi.org/10.1371/journal.pone.0115558 PMID: 25526641.

78. Hayashi Y, Yokota A, Harada H, Huang G. Hypoxia/pseudohypoxia-mediated activation of hypoxia-inducible factor-1α in cancer. Cancer science. 2019; 110(5):1510–7. https://doi.org/10.1111/cas.13990 PMID: 30844107.

79. Wu Y, Yun D, Zhao Y, Wang Y, Sun R, Yan Q, et al. Down regulation of RNA binding motif, single-stranded interacting protein 3, along with up regulation of nuclear HIF1A correlates with poor prognosis in patients with gastric cancer. Oncotarget. 2017; 8(1):1262. https://doi.org/10.18632/oncotarget.13605 PMID: 27902480.

80. Tiwari A, Tashiro K, Dixit A, Soni A, Vogel K, Hall B, et al. Loss of HIF1A from pancreatic cancer cells increases expression of PPP1R1B and degradation of p53 to promote invasion and metastasis. Gastroenterology. 2020; 159(5):1882–97. https://doi.org/10.1053/j.gastro.2020.07.046 PMID: 32768595.

81. Zhang W, Liu K, Pei Y, Tan J, Ma J, Zhao J. Long noncoding RNA HIF1A-AS2 promotes non-small cell lung cancer progression by the miR-153-5p/S100A14 axis. OncoTargets and therapy. 2020; 13:8715. https://doi.org/10.2147/OTT.S262293 PMID: 32922043.

82. Jóźwiak P, Ciesielski P, Zaczek A, Lipińska A, Pomorski L, Wieczorek M, et al. Expression of hypoxia inducible factor 1α and 2α and its association with vitamin C level in thyroid lesions. Journal of Biomedical Science. 2017; 24(1):1–0. https://doi.org/10.1186/s12929-017-0388-y PMID: 29084538.

83. Cucchi DG, Bachas C, van den Heuvel-Eibrink MM, Arentsen-Peters ST, Kwidama ZJ, Schuurhuis GJ, et al. Harnessing gene expression profiles for the identification of ex vivo drug response genes in pediatric acute myeloid leukemia. Cancers. 2020; 12(5):1247. https://doi.org/10.3390/cancers12051247 PMID: 32429253.

84. Su Z, Liu G, Fang T, Zhang K, Yang S, Zhang H, et al. Expression and prognostic value of glutamate dehydrogenase in extrahepatic cholangiocarcinoma patients. American journal of translational research. 2017; 9(5):2106. PMID: 28559964.

85. Zhang Q, Han Q, Yang Z, Ni Y, Agbana YL, Bai H, et al. G6PD facilitates clear cell renal cell carcinoma invasion by enhancing MMP2 expression through ROS-MAPK axis pathway. International journal of oncology. 2020; 57(1):197–212. https://doi.org/10.3892/ijo.2020.5041 PMID: 32319593.

86. Chang HW, Lee M, Lee YS, Kim SH, Lee JC, Park JJ, et al. p53-dependent glutamine usage determines susceptibility to oxidative stress in radioresistant head and neck cancer cells. Cellular Signalling. 2021; 77:109820. https://doi.org/10.1016/j.cellsig.2020.109820 PMID: 33137455.

87. Zeng B, Ye H, Chen J, Cheng D, Cai C, Chen G, et al. LncRNA TUG1 sponges miR-145 to promote cancer progression and regulate glutamine metabolism via Sirt3/GDH axis. Oncotarget. 2017; 8 (69):113650. https://doi.org/10.18632/oncotarget.21922 PMID: 29371936.

88. Yalçın GD, Colak M. SIRT4 prevents excitotoxicity via modulating glutamate metabolism in glioma cells. Human & Experimental Toxicology. 2020; 39(7):938–47. https://doi.org/10.1177/0960327120907142 PMID: 32081049.

89. Michalak S, Rybacka-Mossakowska J, Ambrosius W, Gazdulska J, Gołda-Gocka I, et al. The markers of glutamate metabolism in peripheral blood mononuclear cells and neurological complications in lung cancer patients. Disease Markers. 2016;2016. https://doi.org/10.1155/2016/2895972 PMID: 28044066.

90. Tan Y, Li B, Peng F, Gong G, Li N. Integrative Analysis of Sirtuins and Their Prognostic Significance in Clear Cell Renal Cell Carcinoma. Frontiers in oncology. 2020; 10: 218. https://doi.org/10.3389/fonc.2020.00218 PMID: 32158696

91. Chen X, Xu Z, Zeng S, Wang X, Liu W, Qian L, et al. SIRT5 downregulation is associated with poor prognosis in glioblastoma. Cancer Biomark. (2019) 24:449–59. https://doi.org/10.3233/CBM-182197 PMID: 30909186

92. Zhu Y, Cheng S, Chen S, Zhoa Y. Prognostic and clinicopathological value of SIRT3 expression in various cancers: a systematic review and meta-analysis. OncoTargets and Therapy 2018:11 2157–2167. https://doi.org/10.2147/OTT.S157836 PMID: 29713184

93. Fu W.; Li H.; Fu H.; Zhao S.; Shi W.; Sun M.; et al. The SIRT3 and SIRT6 Promote Prostate Cancer Progression by Inhibiting Necroptosis-Mediated Innate Immune Response. J. Immunol. Res. 2020, 2020, 8820355. https://doi.org/10.1155/2020/8820355 PMID: 33282964

94. Weyemi U, Lagente-Chevallier O, Boufraqech M, Prenois F, Courtin F, Caillou B, et al. ROS-generating NADPH oxidase NOX4 is a critical mediator in oncogenic H-Ras-induced DNA damage and subsequent senescence. Oncogene. 2012; 31(9):1117–29. https://doi.org/10.1038/onc.2011.327 PMID: 21841825.

95. Srinivas US, Tan BW, Vellayappan BA, Jeyasekharan AD. ROS and the DNA damage response in cancer. Redox Biology. 2019; 25: 101084. https://doi.org/10.1016/j.redox.2018.101084 PMID: 30612957.

96. Huang R, Li G, Wang Z, Hu H, Zeng F, Zhang K, et al. Identification of an ATP metabolism-related signature associated with prognosis and immune microenvironment in gliomas. Cancer science. 2020; 111(7):2325–35. https://doi.org/10.1111/cas.14484 PMID: 32415873.

97. Leone RD, Emens LA. Targeting adenosine for cancer immunotherapy. Journal for immunotherapy of cancer. 2018; 6(1):1–9. https://doi.org/10.1186/s40425-018-0360-8 PMID: 29914571.

98. Xu L, Chen J, Jia L, Chen X, Awaleh Moumin F, Cai J. SLC1A3 promotes gastric cancer progression via the PI3K/AKT signalling pathway. Journal of cellular and molecular medicine. 2020; 24(24):14392–404. https://doi.org/10.1111/jcmm.16060 PMID: 33145952.

99. Zińczuk J, Maciejczyk M, Zaręba K, Romaniuk W, Markowski A, Kędra B, et al. Antioxidant barrier, redox status, and oxidative damage to biomolecules in patients with colorectal cancer. Can malondialdehyde and catalase be markers of colorectal cancer advancement?. Biomolecules. 2019; 9(10):637. https://doi.org/10.3390/biom9100637 PMID: 31652642.

100. Yaku K, Okabe K, Hikosaka K, Nakagawa T. NAD metabolism in cancer therapeutics. Frontiers in oncology. 2018; 8:622. https://doi.org/10.3389/fonc.2018.00622 PMID: 30631755.

101. Harris IS, DeNicola GM. The complex interplay between antioxidants and ROS in cancer. Trends in cell biology. 2020; 30(6):440–51. https://doi.org/10.1016/j.tcb.2020.03.002 PMID: 32303435.

102. Yaku K, Okabe K, Nakagawa T. NAD metabolism: Implications in aging and longevity. Ageing research reviews. 2018; 47:1–7. https://doi.org/10.1016/j.arr.2018.05.006 PMID: 29883761.

103. Berger F, Lau C, Dahlmann M, Ziegler M. Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. Journal of Biological Chemistry. 2005; 280(43):36334–41. https://doi.org/10.1074/jbc.M508660200 PMID: 16118205.

104. Zhang T, Berrocal JG, Yao J, DuMond ME, Krishnakumar R, Ruhl DD, et al. Regulation of poly (ADP-ribose) polymerase-1-dependent gene expression through promoter-directed recruitment of a nuclear

NAD+ synthase. Journal of Biological Chemistry. 2012; 287(15):12405–16. https://doi.org/10.1074/jbc.M111.304469 PMID: 22334709.

105. Song T, Yang L, Kabra N, Chen L, Koomen J, Haura EB, et al. The NAD+ synthesis enzyme nicotinamide mononucleotide adenylyltransferase (NMNAT1) regulates ribosomal RNA transcription. Journal of Biological Chemistry. 2013; 288(29):20908–17. https://doi.org/10.1074/jbc.M113.470302 PMID: 23737528.

106. Anderson KA, Madsen AS, Olsen CA, Hirschey MD. Metabolic control by sirtuins and other enzymes that sense NAD+, NADH, or their ratio. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 2017; 1858(12):991–8. https://doi.org/10.1016/j.bbabio.2017.09.005 PMID: 28947253.

107. Heske CM. Beyond energy metabolism: exploiting the additional roles of NAMPT for cancer therapy. Frontiers in oncology. 2020; 9:1514. https://doi.org/10.3389/fonc.2019.01514 PMID: 32010616.

108. Li XQ, Lei J, Mao LH, Wang QL, Xu F, Ran T, et al. NAPRT, Key Enzymes in NAD Salvage Synthesis Pathway, Are of Negative Prognostic Value in Colorectal Cancer. Frontiers Oncology. 2019; 9:736. https://doi.org/10.3389/fonc.2019.00736 PMID: 31448236.