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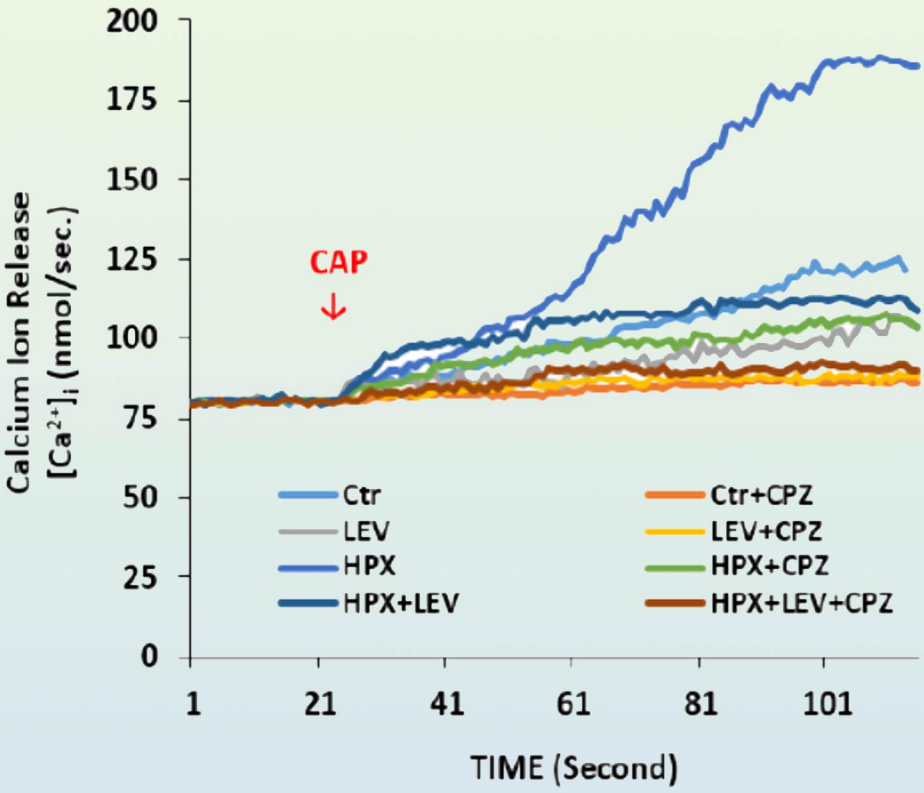


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## [ CONTENTS ]

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**AIM AND SCOPES**
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

**A- Ion Channels** ($Na^+$- $K^+$ Channels, $Cl^-$ channels, $Ca^{2+}$ channels, ADP-Ribose and metabolism of $NAD^+$, Patch-Clamp applications)

**B- Oxidative Stress** (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

**C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience**
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and $NAD^+$ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

**D- Gene and Oxidative Stress**
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

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**Keywords**
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

**RESEARCH ARTICLE**

**J Cell Neurosci Oxid Stress 2019;11(3): 895-902.**

# IDH1 R132H gene mutation reduces cell proliferation and sensitizes recurrent Glioblastoma to hydrogen peroxide

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**Abstract**

Glioblastoma (GBM) recurrence rate is 90% resulting in 15 months median survival only. Isocitrate dehydrogenase 1 (IDH1) mutations in gliomas significantly improved patient's prognosis. Therefore, understanding common IDH1 mutation, *IDH1 R132H* in recurrent GBM is necessary to improve poor survival rate. IDH1 R132H recurrent GBM was developed to investigate cell proliferation rate and sensitivity towards oxidative stress induced by hydrogen peroxide. The cell death mechanism induced by hydrogen peroxide were further investigated. Malaysian recurrent GBM cell line was authenticated via Short Tandem Repeat and screened for *IDH1* gene via PCR. *IDH1 R132H* gene expression in GBM was confirmed via real-time PCR and western blot. The effect of *IDH1 R132H* mutation on cell proliferation rate and cytotoxicity using hydrogen peroxide were determined using MTT assay. The angiogenesis, apoptotic genes and cell cycle induced by hydrogen peroxide in IDH1 wild-type GBM were determined via real-time PCR and flow-cytometry. Malaysian GBM cell line is unique and harbors *IDH1* wild-type gene. *IDH1 R132H* gene mutation significantly reduced the growth rate and sensitized the GBM cells to hydrogen peroxide at 72 hours ($p<0.05$). Hydrogen peroxide induced significant G1 cell cycle arrest and apoptosis in IDH1 wild-type GBM cell line ($p<0.05$). Slower growth rate and higher sensitivity towards oxidative stress may explain why IDH1 mutant patients have better prognosis compared to IDH1 wild-type patients. Confirmation GBM cell death mechanism via hydrogen peroxide showed that it has potential to treat gliomas.

**Keywords**: glioblastoma, IDH1 R132H, proliferation rate, cell cycle, oxidative stress

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**List of Abbreviations;**
***DH1**, Isocitrate dehydrogenase 1; **PCR**, polymerase chain reaction; **GBM**, glioblastoma; **VEGF**, vascular endothelial growth factor; **HIF**, hypoxia inducible factor*

## Introduction

Gliomas are central nervous system tumors developed from glial cells (Mustafa et al. 2013). Annually, about 20,000 people in United State suffered from gliomas (Li et al. 2013). Globocan 2012 estimated that the annual incident of central nervous system (CNS) related tumors in Malaysia to be 2.8 in every 100,000 population with a cumulative rate of 0.3% (Goh et al. 2014). Despite with available treatments, glioblastoma multiforme (GBM) patients have median overall survival of 15 months only (Noushmehr et al. 2010).

*IDH1* gene mutations were first discovered in 12% of GBM via genome-wide sequencing analysis (Parsons et al. 2008). Isocitrate dehydrogenase 1 (*IDH1*) gene is located on chromosome 2q33.3 and localized in peroxisomes and cytoplasm. IDH1 wild-type enzyme catalyzes the conversion of isocitrate to alpha-ketoglutarate (α-KG) and NADPH productions via oxidative decarboxylation (Preusser et al. 2011).

*IDH1* mutations were found in 70-80% of WHO grade II and III diffuse gliomas and secondary GBMs (Gupta et al. 2013). *IDH1* mutations can be used to differentiate gliomas from non-CNS tumors and non-neoplastic conditions (Horbinski et al. 2010) and differentiate secondary GBM from primary GBM, grade II astrocytoma from pilocytic astrocytoma and astrocytoma from ependymomas (Agarwal et al. 2013). In the same tumor grade, glioma patients with IDH1 mutations have better survival compared to *IDH1* wild-type (Boisselier et al. 2010). IDH1 mutant glioma patients were found to have better overall survival (OS) and progression-free disease (PFS) compared to IDH1 wild-type glioma patients (Weller et al. 2009).

There are 7 *IDH1* mutations that have been reported, *IDH1 R132H, R132C, R132S, R132G, R132L, R132V* and *R132P* (Agarwal et al. 2013). *IDH1 R132H* gene mutation was found in approximately 90% of IDH1 mutated gliomas and the remaining IDH1 mutations were less common (Gupta et al. 2013). IDH1 mutant enzymes inhibit the normal enzymatic activity of IDH1 which results in low level of α-KG and acquires new enzymatic activity where α-KG is converted to D-2-hydroxyglutarate (D-2HG) with consumption of NADPH (Loussouarn et al. 2012). D-2HG compete with α-KG to inhibit α-KG dependent dioxygenases such as DNA demethylase and histone demethylase which leads to DNA hypermethylation phenotype (Yang et al. 2012).

In this study, Malaysian established recurrent GBM, USM-Gl-06 cell line (Zawani et al. 2011) was authenticated to confirm the cell line and determine the *IDH1* status. IDH1 R132H protein in *Escherichia coli* was synthesized for future targeted *IDH1 R132H* therapeutic treatment. IDH1 R132H recurrent GBM cell line was used to evaluate the effect of *IDH1 R132H* gene mutation on the recurrent GBM cell's proliferation rate. Since hydrogen peroxide is one of reactive oxygen species (ROS), the effect of recurrent GBM with *IDH1 R132H* gene mutation under oxidative stress was determined using hydrogen peroxide. Our previous finding showed that IDH1 wild-type gliomas were more common compared to IDH1 mutant gliomas at Hospital Universiti Sains Malaysia (Goh et al. 2019). Since IDH1 wild-type gliomas more common, IDH1 wild-type recurrent GBM cells was used to further elucidate the cell death mechanism induced by hydrogen peroxide.

To our current knowledge, this is the first study to show IDH1 R132H mutant recurrent GBM was sensitive to hydrogen peroxide compared to the IDH1 wild-type recurrent GBM. Hydrogen peroxide able to induce G1 phase cell cycle arrest, forcing the IDH1 wild-type recurrent GBM cells into apoptosis. Therefore, any substances that can produce hydrogen peroxide may have potential in glioma chemotherapy.

## Materials and Methods

### Cell line

Human recurrent glioblastoma cell line, USM-Gl-06 was established from Hospital Universiti Sains Malaysia recurrent glioblastoma patient (Zawani et al. 2011). The cell line was maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (Invitrogen), and 1% penicillin/streptomycin in a 37°C, humidified and 5% $CO_2$ incubator.

### Cell line authentication and IDH1 screening of USM-Gl-06

Genomic DNA from the cell line was subjected to short tandem repeat (STR) profiling using AmpF/STR Identifiler Direct PCR Amplification kit (ThermoFisher Scientific). Fifteen STR loci and gender determination locus, Amelogenin were amplified and compared to ATCC STR cell line database. Hotspot mutation, codon 132 in *IDH1* gene was PCR amplified (Meyer et al. 2010)

using KOD hot start polymerase (Toyobo) and sequenced by Apical Scientific, Malaysia.

**Construction of IDH1 R132H mammalian plasmid**

*IDH1* wild-type gene was isolated from the cell line via reverse-transcription PCR using OneTaq One-Step RT-PCR Kit (New England Biolabs) and cloned into pET-47b(+) plasmid. Primers used for IDH1 gene isolation are 3'ATGTCCAAAAAAATCAGTGG-CGGT5' and 3'TTAAAGTTTGGCCTGAGCTAGT5'. The pET-47b(+) IDH1 wild-type plasmid was used as template to mutate into *IDH1 R132H* at codon 132 via QuikChange II XL Site-Directed Mutagenesis Kit (Agilent) using 3'GTAAAACCTATCATCATAGGTC-ATCATGCTTATGGGGATCAATAC5' and 3'GTATT-GATCCCCATAAGCATGATGACCTATGATGATAGG TTTTAC5' primers. *IDH1 R132H* gene was further cloned into pEGFP-C1 plasmid generating pEGFP-C1 IDH1 R132H mammalian plasmid.

**Generation of IDH1 R132H USM-Gl-06 mutant cell line**

Prior to transfection, optimum G418 (Invitrogen) concentration was determined in 50,000 cells for 14 days. pEGFP-C1 IDH1 R132H plasmid was transfected into the cell line and maintained in RPMI medium with 100µg/ml G418 using jetPRIME (Polyplus). The survived cells after G418 selection were diluted to a single cell in 96-well plate and cultured until confluent in T75 flask. The IDH1 R132H protein was confirmed via Western blot. IDH1 qPCR primers; 3'ACATGGTGGCCCAAGCTA5' and 3'AGCAATGGGATTGGTGGA5' and beta-actin (ACTB) qPCR primers; 3'AGAGCTACGAG-CTGCCTGAC5' and 3'AGCACTGTGTTGGCGT-ACAG5' were used for *IDH1* gene quantification via Luna Universal One-Step RT-qPCR Kit (New England Biolab).

**Cell proliferation assay**

IDH1 wild-type USM-Gl-06 and IDH1 R132H USM-Gl-06 cell lines were seeded at a density of 4,000 cells per well in 96-well culture plates. The number of cells were determined using MTT assay (ThermoFisher Scientific) at 570 nm wavelength using Model 680 Microplate Reader (Biorad). The experiments were performed for 24 hours, 48 hours and 72 hours respectively. The significant differences between the two cell lines at each time point were analyzed.

**Cytotoxicity assay using hydrogen peroxide ($H_2O_2$)**

Approximately 5 x $10^4$ cells of IDH1 wild-type USM-Gl-06 and IDH1 R132H USM-Gl-06 were seeded per well in 96-well plate and allowed to grow until 80% confluent. RPMI medium with 2-fold dilutions of hydrogen peroxide concentrations were incubated for 72 hour ranging from 2000 µM, 1000 µM, 500 µM, 250 µM, 125 µM, 62.5 µM, 31.3 µM, 15.6 µM, 7.8 µM, 3.9 µM to 0 µM. After incubation, the cell viability were determined using MTT assay as previously described. Half maximal inhibitory concentration (IC50) was determined from dose-response curve. The significant differences of IC50 between the IDH1 wild-type USM-Gl-06 and IDH1 R132H USM-Gl-06 mutant cells were analyzed.

**Bax, Bcl-2, HIF-1α and VEGF-A genes quantification**

Genes expression involved in angiogenesis, *HIF-1α* and *VEGF-A* and apoptosis, *Bax* and *Bcl-2*, were determined by real-time PCR analysis as previously described. The primer sequences were as follows: *Bcl-2* 3'ATGTGTGTGGAGAGCGTCAA5' and 3'ACAGTT-CCACAAAGGCATCC5', *Bax* 3'TTGCTTCAGGGTTT-CATCCA5' and 3'CAGCCTTGAGCACCAGTTTG5', *HIF-1α* 3'AAGTCTGCAACATGGAAGGTAT5' and 3'TGAGGAATGGGTTCACAAATC5', *VEGF-A* 3'AA-GGAGGAGGGCAGAATCAT5' and 3' ATCTGCAT-GGTGATGTTGGA5'. The mRNA expression was expressed as fold-change relative to the untreated USM-Gl-06 cell line as control.

**Cell Cycle Analysis Assay**

Approximately 0.3x$10^6$ USM-Gl-06 cells were seeded in a 6-well plate. After 80% confluent, the cells were treated with IC50 hydrogen peroxide concentrations, 307.6 µM. After 72 hours incubation, cells were collected and fixed with ice-cold 70% ethanol for 12 hours at 4$^o$C. Cells were stained with propidium iodide and analyzed using Flow Cytometry (BD FACSCalibur). Approximately 2x$10^4$ cells were analyzed, cell debris and clumps were excluded in the analysis.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism version 6 (GraphPad Software). The results were presented as mean ± standard error mean (SEM). The

significance differences between the means were analyzed using independent t-test. All the statistical analysis were performed at the significance level of $p < 0.05$.

## Results

### USM-Gl-06 cell line is unique and harbors IDH1 wild-type gene

Eight STR genes of the USM-Gl-06 cell line (Table 1) showed that the cell line is unique compared to available cell lines in the ATCC STR database. Amplified 500bp PCR amplicon was sequenced to determine the nucleotides at codon 132 as shown in the electropherogram results (Figure 1). The nucleotides at codon 132 were found to be CGT (Arginine) which is *IDH1* wild-type gene.

| Genetic Locus | USM-Gl-06 |
|---|---|
| Amelogenin | X,X |
| D7S820 | 11 |
| CSF1PO | 10,11 |
| TH01 | 8 |
| D13S317 | 9 |
| D16S539 | 12 |
| vWA | 15 |
| TPOX | 8 |
| D5S818 | 12 |

***Table 1:*** *Short Tandem Repeat profile of USM-Gl-06 cell line*

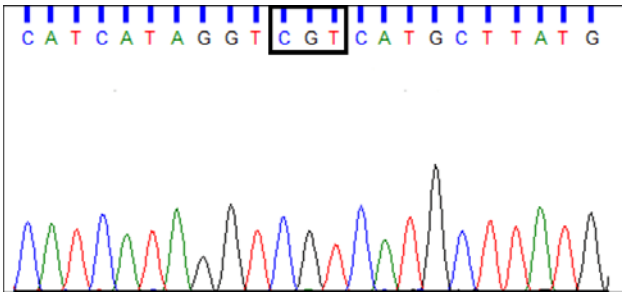


***Figure 1. Electropherogram result of IDH1 gene in USM-Gl-06 cell line.*** *The box indicates wild-type nucleotides (CGT) at codon 132.*

### IDH1 R132H USM-Gl-06 mutant cell line was successfully generated

IDH1 R132H protein in the transfected cell line was confirmed via Western assay (Figure 2) and significant overexpression of *IDH1* gene was confirmed using real-time PCR ($p<0.01$) (Figure 3)

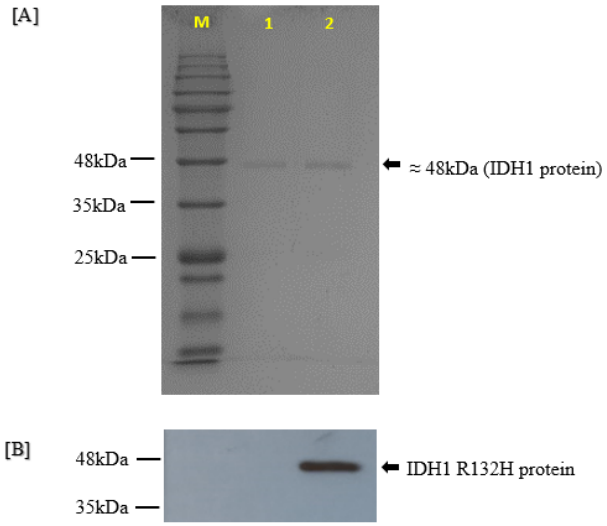


***Figure 2.*** *Confirmation of IDH1 R132H mutant and β-actin proteins in stably transfected IDH1 R132H USM-Gl-06 mutant cell lysate. [A] Lane 1: Untransfected cell lysate, Lane 2: Stable transfected cell lysate, Lane 3: Purified IDH1 R132H recombinant protein from E.coli. The membrane was detected using monoclonal anti-IDH1 R132H antibody. [B] β-actin in both untransfected and transfected USM-Gl-06 cell lysate were successfully detected using polyclonal anti-β actin antibody.*

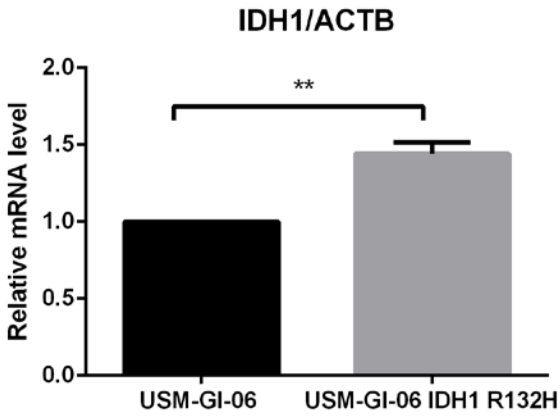


***Figure 3.*** *Relative fold change of IDH1 mRNA levels in transfected IDH1 R132H USM-Gl-06 mutant cell line normalized with (ACTB) β-actin. Error bars are mean ± standard error mean (SEM) from three replicates. Asterisk (**) indicates significance between column ($p<0.01$) where $p = 0.0037$ (independent t test).*

**IDH1 R132H gene mutation induce slower cell proliferation in recurrent GBM**

The IDH1 R132H recurrent GBM cell line showed slower cell proliferation from 48 hours onwards. The proliferation rate of mutant cells was significantly slower compared to the IDH1 wild-type recurrent GBM cells at 72 hours ($p$<0.01) (Figure 4).

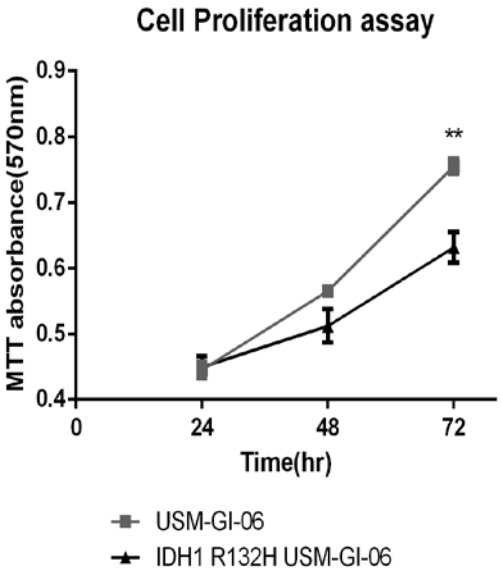


***Figure 4:*** *Cell proliferation rate between IDH1 wild-type USM-Gl-06 and IDH1 R132H USM-Gl-06. Error bars are mean ± standard error mean (SEM) from 3 biological replicates. Asterisk (**) indicates significance between column (p<0.01) where p= 0.0091 (independent t test). The IDH1 R132H USM-Gl-06 mutant cells showed a significant slower cell proliferation rate compared to IDH1 wild-type USM-Gl-06 cells at 72 hour (p<0.01).*

**IDH1 R132H gene mutation sensitizes recurrent GBM cell to oxidative stress**

Hydrogen peroxide is one of the reactive oxygen species (ROS) (Pelicano et al. 2004). Therefore, it was used to induce oxidative stress in the cells. It was found that USM-Gl-06 transfected with *IDH1 R132H* gene mutation significantly lower the IC50, 233.3 µM compared to the IDH1 wild-type GBM cell line, 307.6 µM ($p$<0.05) (Figure 5 and Figure 6).

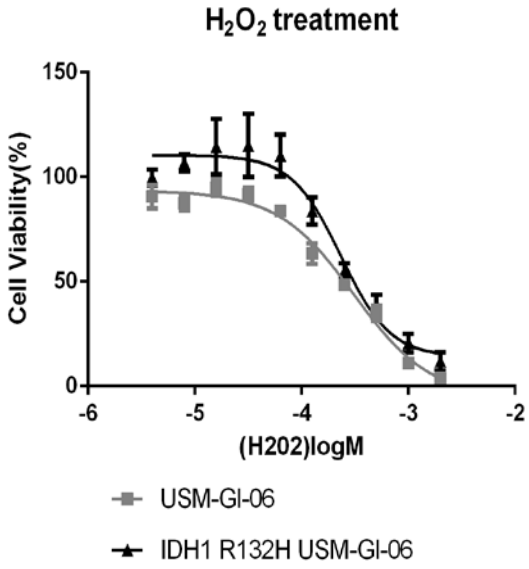


**Figure 5:** Percentage of cell viability between IDH1 wild-type USM-Gl-06 and IDH1 R132H USM-Gl-06 at different concentration of hydrogen peroxide (H2O2) at 72 hours. Error bars are mean ± standard error mean (SEM) from 3 biological replicates. IDH1 R132H USM-Gl-06 showed a lower IC50, 233.3 µM compared to the IDH1 wild-type USM-Gl-06, 307.6 µM.

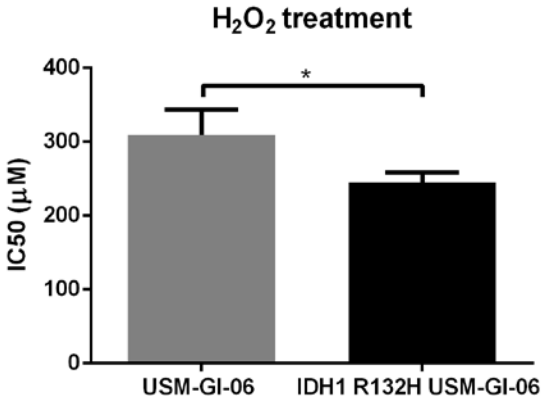


***Figure 6:*** *Comparison of IC50 between IDH1 wild-type USM-Gl-06 and IDH1 R132H USM-Gl-06 cell line. Error bars are mean ± standard error mean (SEM) from three replicates. Asterisk (*) indicates significance between column (p<0.05) where p = 0.0401 (independent t test). IDH1 R132H USM-Gl-06 mutant cells significantly sensitive to hydrogen peroxide compared to IDH1 wild-type cells.*

**Hydrogen peroxide induces apoptosis in recurrent GBM.**

After hydrogen peroxide treatment, hypoxia-inducible 1 alpha (*HIF-1α*) and *Bax* genes were upregulated but *Bcl-2* downregulated significantly ($p$<0.05). *VEGF-A* gene did showed upregulation but there is no significance. It was observed that the fold-

change of *Bax* gene expression overwhelm the *HIF-1α* gene (Figure 7).

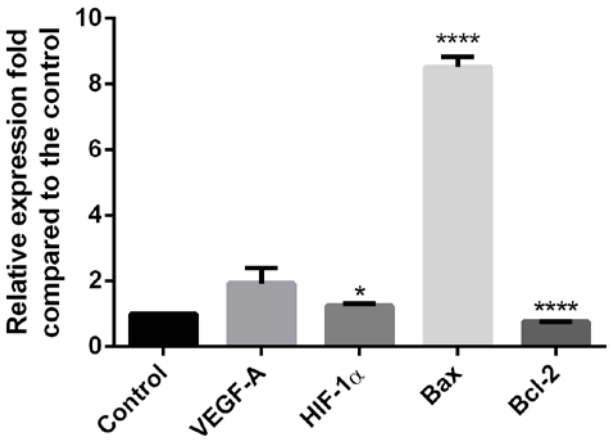


***Figure 7.*** *Relative gene expression of VEGF-A, HIF-1α, Bax and Bcl-2 in USM-Gl-06 cells treated with 307.6 µM hydrogen peroxide at 72 hours compared to untreated USM-Gl-06 cells as control. Error bars are mean ± standard error mean (SEM) from three replicates. Asterisk (*) indicates the significance between columns (p<0.05) and (****) indicates (p<0.0001) (independent t test).*

**Hydrogen peroxide induces G1 Phase Arrest in recurrent GBM.**

The cell death mechanism by hydrogen peroxide on glioma cell cycle progression was determined. IDH1 wild-type USM-Gl-06 cells were treated with IC50 concentration, 307.6 µM of hydrogen peroxide and analyzed via flow cytometry. Results showed that 307.6 µM of hydrogen peroxide caused a significant glioma cell arrest at G1 phase ($p$<0.001) (Figure 8C).

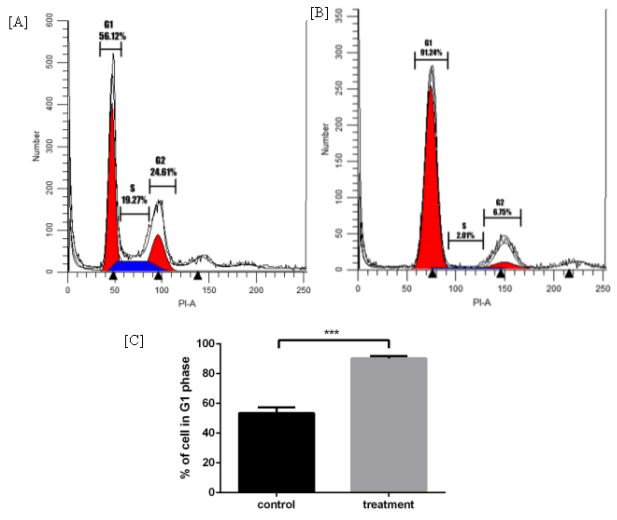


***Figure 8.*** *[A] and [B] shows representative cell cycle profile of untreated USM-Gl-06 cells as control and USM-Gl-06 cells treated with 307.6 µM hydrogen peroxide at 72 hours respectively. [C] shows the percentage of cells in G1 phase treated with 307.6 µM hydrogen peroxide compared to untreated control for 72 hours. Error bars are standard error mean (SEM) from three replicates. Asterisk (***) indicates the significance between columns (p<0.001) (independent t test).*

**Discussion**

The current treatments for glioma are surgery, radiation and chemotherapy. Despite all these, the recurrence of GBM is almost certain (van Linde et al. 2017). It was evident that recurrent GBM developed resistance towards the previous treatment regimen (Lau et al. 2014). Therefore, the dosage and frequency of treatments need to be increased to kill the cancer cells. However, this will increase neurotoxicity burden in patient's body.

Malaysian established recurrent GBM cell line, USM-Gl-06 GBM cell line was found to harbor *IDH1* wild-type gene. Based on the Short Tandem Repeat (STR) analysis, this cell line was proven unique compared to other available cell lines in the ATCC database. As the prognosis of IDH1 wild-type recurrent GBM patients is poor, USM-Gl-06 can serve as a new model to represent Malaysian IDH1 wild-type recurrent GBM patients for drug testing and fundamental study.

Bevacizumab is the only monoclonal antibody approved by the Food and Drug Administration (FDA) for recurrent GBM treatment targeting vascular endothelial growth factor (VEGF) (Lau et al. 2014). However, it was found unable to prolong overall survival (OS) in recurrent and newly diagnosed GBMs (van Linde et al. 2017). Since IDH1 mutations were found highly in glioma patients (Hartmann et al. 2009), this gene can be utilized to enhance current treatments, treatment stratification or development of new alternative therapy.

Glioma with natural existing IDH1 R132H was proven failed to culture ex vivo (Piaskowski et al. 2011). Thus, stable IDH1 R132H USM-Gl-06 cell line was established via transfection to study the effect of *IDH1 R132H* mutation and confirmed via Western blot and real-time PCR ($p$<0.01). IDH1 R132H inhibits α-KG production by IDH1 wild-type and further converts α-KG into D-enantiomer hydroxyglutarate (D-2HG) (Pusch et al. 2014). The presence of IDH1 R132H mutant enzyme in transfected GBM cells lowered the α-KG level. Since

α-KG is one of substrates in Krebs cycle, therefore the energy production in mitochondria was affected. Low energy supply in the mutant cells results in slower proliferation rate compared to the IDH1 wild-type GBM cells ($p<0.01$).

IDH1 R132H inhibits IDH1 wild-type enzyme to generate NADPH and promotes NADPH oxidation which further lower NADPH level in the cells (Li et al. 2013). NADPH is important for maintaining redox balance in the cells (Bhattacharya 2015). Hydrogen peroxide is one of reactive oxygen species and associated with cancer cell killing in common chemotherapeutic drugs such as cisplatin, paclitaxel, etoposide and doxorubicin (Lopez-Lazaro 2007). However, killing of glioma cells using hydrogen peroxide is yet to be elucidated.

Theoretically, low level of NADPH disrupts the redox balance and sensitized mutant cells towards oxidative stress. IDH1 mutant glioma cells was postulated unable to overcome the oxidative stress induced by hydrogen peroxide ($H_2O_2$) which leads to cell death. However, IDH1 wild-type glioma cells have normal NAPDH level to buffer the oxidative stress resulting in cell survival. In this study, we showed that the IDH1 R132H mutant glioma cells were significantly sensitive towards $H_2O_2$, IC50 value 233.3 µM compared to the IDH1 wild-type glioma cells, 307.6 µM ($p<0.05$).

Previous study of *IDH1* genetic screening at Hospital Universiti Sains Malaysia showed that IDH1 wild-type glioma patients constituted of 93.6% whereas the remaining were IDH1 mutants (Goh et al. 2019). Due to high prevalence of *IDH1* wild-type in gliomas, the mechanism cell death induced by hydrogen peroxide in IDH1 wild-type USM-Gl-06 was further investigated targeting apoptosis and angiogenesis genes. Results showed that *HIF-1α* and *Bax* genes were significantly upregulated ($p<0.05$). Although *HIF-1α* involved in angiogenesis (Fu et al. 2012), higher expression of pro-apoptotic protein, Bax (Ayyagari et al. 2017) overwhelm the HIF-1α effect resulting in glioma cell apoptosis. Besides that, the effect of glioma angiogenesis cannot be elucidate in the current study. Further study needs to be conducted in animal model to confirm this finding.

Hydrogen peroxide able to inhibit the USM-Gl-06 cells growth via G1 phase cell cycle arrest. Cell cycle checkpoints help to protect mitotic cells from DNA damage that will leads to cancer development. When the cell's DNA damaged, cells are arrested in G1 phase to prevent the cells entering S phase for DNA repair. Various chemotherapeutic compounds were found able to kill cancers by arresting G1 phase such as esculetin in prostate cancer (Turkekul et al. 2018), piperine in melanoma (Fofaria et al. 2014) and 3-Nitroacridine derivatives in breast cancer (Zhou et al. 2018). Prolong exposure to hydrogen peroxide may force the cells into apoptosis which was confirmed by high *Bax* and low *Bcl-2* gene expression ($p<0.0001$).

In conclusion, confirmation of USM-Gl-06 cell line showed that this cell line is an alternative glioma model and may closer to Asian genetic makeup compared to the commercially available Caucasian glioma cell line. To our current knowledge, this is the first study that showed IDH1 R132H mutant recurrent GBM was sensitive to hydrogen peroxide compared to the IDH1 wild-type GBM. In addition, hydrogen peroxide can induce G1 phase cell cycle arrest, forcing the glioma cell into apoptosis. Therefore, substances that produce hydrogen peroxide may have potential in glioma chemotherapy treatment. This study also showed that *IDH1* status is important in determining the effective drug dosage to reduce neurotoxicity burden and improving overall survival rate.

**Acknowledgment**

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**Conflict of interest**

The authors declare no conflicts of interest.

**References**

Agarwal S, Sharma MC, Jha P, Pathak P, Suri V. 2013. Comparative study of IDH1 mutations in gliomas by immunohistochemistry and DNA sequencing. *Neuro. Oncol.* 15(6):718–26

Ayyagari VN, Diaz-Sylvester PL, Hsieh THJ, Brard L. 2017. Evaluation of the cytotoxicity of the Bithionol-paclitaxel combination in a panel of human ovarian cancer cell lines. *PLoS One.* 12(9):e0185111

Bhattacharya S. 2015. Reactive Oxygen Species and Cellular Defense System. *Free Radicals Hum. Heal. Dis.* 17–29

Boisselier B, Marie Y, Labussière M, Ciccarino P, Desestret V, et al. 2010. COLD PCR HRM: A highly sensitive detection method for IDH1 mutations. *Hum. Mutat.* 31(12):1360–65

Fofaria NM, Kim S, Srivastava SK. 2014. Piperine Causes G1 Phase Cell Cycle Arrest and Apoptosis in Melanoma Cells through Checkpoint Kinase-1 Activation. *PLoS One*. 9(5):1–10

Fu Y, Zheng S, Zheng Y, Huang R, An N, et al. 2012. Glioma derived

isocitrate dehydrogenase-2 mutations induced up-regulation of HIF-1α and β-catenin signaling: Possible impact on glioma cell metastasis and chemo-resistance. *Int. J. Biochem. Cell Biol.* 44(5):770–75

Goh CH, Lu YY, Lau BL, Wong JOL, Lee HK, et al. 2014. Brain and spinal tumour. *Med. J. Malaysia*. 69(6):261–67

Goh WC, Idris B, Kandasamy R, Shamsuddin S, Jaafar H. 2019. PCR-RFLP method enhance DNA sequencing of IDH1 somatic mutations detection in gliomas. *Gulhane Med. J.* 61(4):167–71

Gupta R, Flanagan S, Li CC, Lee M, Shivalingham B, et al. 2013. Expanding the spectrum of IDH1 mutations in gliomas. *Mod. Pathol.* 26(5):619–25

Hartmann C, Meyer J, Balss J, Capper D, Mueller W, et al. 2009. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: A study of 1,010 diffuse gliomas. *Acta Neuropathol.* 118(4):469–74

Horbinski C, Kelly L, Nikiforov YE, Durso MB, Nikiforova MN. 2010. Detection of IDH1 and IDH2 mutations by fluorescence melting curve analysis as a diagnostic tool for brain biopsies. *J. Mol. Diagnostics.* 12(4):487–92

Lau D, Magill ST, Aghi MK. 2014. Molecularly targeted therapies for recurrent glioblastoma: Current and future targets. *Neurosurg. Focus.* 37(6):

Li S, Chou AP, Chen W, Chen R, Deng Y, et al. 2013. Overexpression of isocitrate dehydrogenase mutant proteins renders glioma cells more sensitive to radiation. *Neuro. Oncol.* 15(1):57–68

Lopez-Lazaro M. 2007. Dual role of hydrogen peroxide in cancer : Possible relevance to cancer chemoprevention and therapy. *Cancer Lett.* 252:1–8

Loussouarn D, Le Loupp AG, Frenel JS, Leclair F, Von Deimling A, et al. 2012. Comparison of immunohistochemistry, DNA sequencing and allele-specific PCR for the detection of IDH1 mutations in gliomas. *Int. J. Oncol.* 40(6):2058–62

Meyer J, Pusch S, Balss J, Capper D, Mueller W, et al. 2010. PCR- and restriction endonuclease-based detection of IDH1 mutations. *Brain Pathol*. 20(2):298–300

Mustafa Z, Shamsuddin HS, Ideris A, Ibrahim R, Jaafar H, et al. 2013. Viability reduction and rac1 gene downregulation of heterogeneous Ex-Vivo glioma acute slice infected by the oncolytic newcastle disease virus strain V4UPM. *Biomed Res. Int.* 2013:

Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Berman BP, et al. 2010. Identification of a CpG Island Methylator Phenotype that Defines a Distinct Subgroup of Glioma. *Cancer Cell*. 17(5):510–22

Parsons DW, Jones S, Zhang X, Lin JC-H, Leary RJ, et al. 2008. An integrated genomic analysis of human glioblastoma multiforme. *Science (80-. ).* 321(5897):1807–12

Pelicano H, Carney D, Huang P. 2004. ROS stress in cancer cells and therapeutic implications. *Drug Resist. Updat.* 7(2):97–110

Piaskowski S, Bienkowski M, Stoczynska-Fidelus E, Stawski R, Sieruta M, et al. 2011. Glioma cells showing IDH1 mutation cannot be propagated in standard cell culture conditions. *Br. J. Cancer*. 104(6):968–70

Preusser M, Wohrer A, Stary S, Hoftberger R, Streubel B, Hainfellner JA. 2011. Value and limitations of immunohistochemistry and gene sequencing for detection of the IDH1-R132H mutation in diffuse glioma biopsy specimens. *J Neuropathol Exp Neurol*. 70(8):715–23

Pusch S, Schweizer L, Beck AC, Lehmler JM, Weissert S, et al. 2014. D-2-Hydroxyglutarate producing neo-enzymatic activity inversely correlates with frequency of the type of isocitrate dehydrogenase 1 mutations found in glioma. *Acta Neuropathol. Commun.* 2(19):1–10

Turkekul K, Colpan RD, Baykul T, Ozdemir MD, Erdogan S. 2018. Esculetin Inhibits the Survival of Human Prostate Cancer Cells by Inducing Apoptosis and Arresting the Cell Cycle. *J. Cancer Prev.* 23(1):10–17

van Linde ME, Brahm CG, de Witt Hamer PC, Reijneveld JC, Bruynzeel AME, et al. 2017. Treatment outcome of patients with recurrent glioblastoma multiforme: a retrospective multicenter analysis. *J. Neurooncol.* 135(1):183–92

Weller M, Felsberg J, Hartmann C, Berger H, Steinbach JP, et al. 2009. Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: A prospective translational study of the German Glioma Network. *J. Clin. Oncol.* 27(34):5743–50

Yang H, Ye D, Guan KL, Xiong Y. 2012. IDH1 and IDH2 mutations in tumorigenesis: Mechanistic insights and clinical perspectives. *Clin. Cancer Res.* 18(20):5562–71

Zawani S, Ramli M, Shamsuddin S, Hassan NN. 2011. The Expression of BORIS Protein in a Newly Established Primary Glioma Cell Culture Line. *Mol. Targets CNS Tumors*

Zhou Q, You C, Zheng C, Gu Y, Gu H, et al. 2018. 3-Nitroacridine derivatives arrest cell cycle at G0/G1 phase and induce apoptosis in human breast cancer cells may act as DNA-target anticancer agents. *Life Sci.* 206(2017):1–9