Significance of tumor protein p53 mutation in cellular process and drug selection in brain lower grade (WHO grades II and III) glioma

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Aim: Tumor protein p53 (*TP53*) mutant is one of the most frequently mutated genes in glioma. **Results:** The Cancer Genome Atlas data has shown that *TP53* mutation is present in 49% of lower grade (World Health Organization [WHO] grades II and III) glioma patients. Data from The Genomics of Drug Sensitivity in Cancer database showed that three drugs: (5Z)-7-oxozeaenol, dabrafenib and nutlin-3a (–), have shown more resistance in patients with *TP53* mutation. We identified 1100 differentially expressed genes. Functional enrichment analysis showed that the differentially expressed genes are mainly concentrated in the transport of ionic and cancer-related pathways. The top ten hub genes were identified and an outcome analysis revealed the most critical genes related to prognosis. **Conclusion:** Our results identified the key genes and pathways that might provide the basic proof to improve individualized treatment in patients with glioma.

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Keywords: bioinformatics analysis • brain lower grade glioma • RNA sequencing • TCGA • *TP53* mutation

Glial cells are the main cells which support the brain's nerve cells and keep them healthy. As we all know, glioma is a cancer that develops in the brain's glial cells [1]. Glioma is the most common CNS tumor and is prevalent in about half of all intracranial primary tumors, and all neuroepithelial-derived tumors. It refers to all tumors originating from various types of glial cells. In 2016, the World Health Organization (WHO) established a new concept of molecular diagnosis of such tumors, which were divided into oligodendroglioma with a *IDH* mutation, as well as a 1p/19q codeletion, diffuse astrocytoma with a *IDH* mutation, anaplastic oligodendroglioma (*IDH*-mutated and 1p/19q codeletion), *IDH*-mutated glioblastoma, *IDH*-mutated anaplastic astrocytoma, *IDH* wild-type glioblastoma, diffuse midline glioma with H3K27M mutation and so on. Moreover, tumors are classified clinically as grade I, II, III or IV according to the standards set by the WHO [2]. Based on this classification, low- and intermediate-grade gliomas (WHO II and III) are also called lower-grade gliomas (LRGGs), which have highly variable clinical behaviors that are not adequately predicted on the basis of histologic class. In this study, we focus on these LRGGs [3–5]. Despite the grade, glioma will compress normal brain tissue and cause pressure and space related disabilities or fatal consequences as it grows in size. In 2010, more than 22,000 Americans were estimated to be diagnosed, and an estimated 13,140 people have died of complications related to brain and other neurological cancers [6].

Nowadays, the choice of individualized therapy is currently based on the results of molecular pathological analysis and individualized drug testing. These tests can help doctors choose more suitable chemotherapeutic agents based on the different characteristics of patients' tumor cells for personalized treatment, which could significantly delay disease progression and improve prognosis. In summary, the ability to determine tumor characteristics of glioma patients can provide a theoretical basis for better-individualized treatment.

TP53 is a well-known tumor suppressor that plays a central role in many tumors [7]. It can play multiple roles in complex cellular processes by regulating the expression of target genes [8]. A mutation in *TP53* is found in multiple cancer types, including glioma [9]. Previous results indicated that almost 50% of the glioma samples had a *TP53* mutation [10]. A *TP53* mutation can promote the disease program and affect treatment strategies [11,12]. Many previous studies have shown that mutant *TP53* can accelerate cell proliferation and promote cancer cell

metastasis [13]. Since the *TP53* mutation is one of the most commonly detected mutations in glioma patients, we plan to explore whether we can define glioma patients with a *TP53* mutation as a subgroup, thus helping identify some novel personalized treatment methods.

In this study, we used the data in the Genomics of Drug Sensitivity in Cancer (GDSC) database to assess the significance of *TP53* mutations in the selection of chemotherapy drugs for glioma. In addition, the RNA sequencing (RNA-seq) data of brain LRGG was downloaded and analyzed to explore the role of *TP53* mutation in disease progression, thereby providing novel personalized treatment options.

Materials & methods

RNA-seq data

We downloaded brain LRGG RNA-seq data directly from The Cancer Genome Atlas (TCGA) database. Then we obtained the corresponding clinical information from the cBioPortal for the Cancer Genomics website (Brain LRGG, TCGA and the PanCancer Atlas) [14].

Analysis of the GDSC database

The GDSC project is mainly used for cancer molecular treatment and mutation exploration [15]. Public online platforms have been developed to help researchers analyze and download relevant data [16]. In this study, we searched for the compounds with selectivity for *TP53* mutation at first. Then we searched for tumor chemotherapy drugs specific to LRGG cells and downloaded the volcano plots and scatter plots directly from the GDSC online platform.

Gene set enrichment analysis

We analyzed the genetic differences of biological annotations and pathways in gene set enrichment analysis (GSEA) to help us further understand the effect of *TP53* mutations on the biological function of patients with LRGG. We downloaded the GSEA 4.0.3 software directly from the Broad Institute website (<https://www.gsea-msigdb.org/gsea/index.jsp>). Then we imported the data matrix in the software and the number of permutations was set at 100. H.all.v7.1.symbols.gmt was chosen as the gene sets database. Enrichment results satisfying the p -value < 0.05 with a false discovery rate < 0.25 were considered statistically significant.

Identification of differentially expressed genes

We divided the 508 LRGG patients from TCGA into a *TP53* mutant group and a *TP53* wild-type group according to whether *TP53* was mutated. We used EdgeR to find out differentially expressed genes (DEGs) between *TP53* mutation and wild-type groups [17,18]. Genes with fold change ≥ 2 or ≤ 0.5 and the p -value < 0.05 were identified as DEGs.

Gene ontology & pathway enrichment analysis of DEGs

In this study, the database for annotation, visualization and integrated discovery (DAVID; <https://david.ncifcrf.gov/>) was employed to deal with gene functional analysis [19]. In this section, we mainly focused on gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analysis. All the DEGs were uploaded to the DAVID website for enrichment analysis, while specifying a $p < 0.05$ for statistical significance.

Analyses of protein–protein interaction networks & module

A search tool for the retrieval of interacting genes (STRING) database provides a critical assessment and integration of protein–protein interaction (PPI) for researchers to analyze protein interaction [20]. We used this database in the analysis of PPI. To evaluate the interaction among all the DEGs we have identified, we submitted all the DEGs to the STRING website. Then, only experimentally validated interactions (total score > 0.4) were selected as meaningful. The PPI network was then used for module screening by molecular complex detection in Cytoscape (scores > 3 , nodes > 4) [21]. The hub genes, which are highly interconnected with the nodes in the module, have shown important functions. Nodes with a connection degree > 10 were selected as hub genes.

Statistical analyses

We used student t -tests to compare gene mRNA expression data in *TP53* mutants and wild-type LRGG tissues. As for the prognosis analysis, expression levels of the selected hub genes were divided into high- or low-expression levels according to the median value at the cut-off point. Then, the Kaplan–Meier method and log-rank test using

Figure 1. Mutation frequency and types of tumor protein p53 in lower grade glioma from The Cancer Genome Atlas database. (A) Mutation frequency and mutation types of *TP53* in lower grade glioma. **(B)** Figure showed where missense mutations and truncating mutations locate on the protein. TAD: Transactivation motif; Tetramer: Tetramerization motif.

Graphpad were used to calculate clinical prognosis. All statistical analyses were performed using Graphpad. $p < 0.05$ was considered statistically significant.

Results

TP53 mutation is highly detected in LRGG

We downloaded tumor tissue gene expression data and completed clinical follow-up data of 508 patients with LRGG from the TCGA database. Of all patients, 250 patients had *TP53* mutation, accounting for approximately 49% of the total (Figure 1A). In the cases of *TP53* mutation, the types of mutations include amplification, truncation, deep deletion, in-frame mutations and missense mutations that span the entire gene (Figure 1B).

TP53 mutation does not influence LRGG prognosis

Next, we explored the impact of *TP53* mutations on prognosis and treatment. We first investigated *TP53* mRNA expression in *TP53* wild-type and mutated groups. Our results showed that there was no significant difference in *TP53* expression levels between the two groups (Figure 2A). Additionally, we identified that the *TP53* mutation was found to have no effect on disease prognosis (Figure 2B).

LRGG cells with *TP53* mutation are resistant to (5Z)-7-oxozeaenol, dabrafenib & nutlin-3a (–)

The sensitivity and resistance to chemotherapeutic drugs determines the outcome of therapy. We further investigated whether the *TP53* mutation has any impact on the increase or decrease in the sensitivity or resistance of chemotherapeutic drugs in LRGG. We queried the GDSC database to determine whether the *TP53* mutant cells had specific therapeutic drugs or compounds. The results suggested that four drugs: (5Z)-7-oxozeaenol (OZ), bleomycin, dabrafenib and nutlin-3a (–) were both resistant in cancer cells with a *TP53* mutation (Figure 3). Furthermore, we explored the drug resistance or sensitivity in LRGG cells, and found that LRGG cells that harbor a *TP53* mutation, were significantly resistance to only (5Z)-7-OZ, dabrafenib and nutlin-3a (–) (Figure 4). We discovered that for LRGG patients with *TP53* mutation, (5Z)-7-OZ, dabrafenib and nutlin-3a (–) may not be therapeutically suitable compounds.

Results of GSEA

Our results indicated that the *TP53* mutation plays a critical role in drug selection for LRGG. We then analyzed the effects of *TP53* mutations on cellular processes. We first analyzed the GSEA method to deal with biological

Figure 2. Mutation of tumor protein p53 and lower grade glioma prognosis. (A) Correlation between *TP53* mutation and mRNA expression. **(B)** Kaplan–Meier survival curves for lower grade glioma patients stratified by *TP53* mutation. Log-rank test was used for analysis and a p-value < 0.05 for statistical significance. NS: Not significant.

functional gene sets. The results in Figure 5 indicated that *TGB-β* signaling, *NOTCH* signaling and mitotic spindles were significantly enriched, which suggests that the *TP53* mutation may influence LRGG progression and drug selection, by influencing the aforementioned pathways and cell division processes.

Identification of DEGs

Next, we identified DEGs in gene expression data to find pathways and genes related to a *TP53* mutation. We divided the 508 LRGG patients into a *TP53* mutant group and a *TP53* wild-type group according to whether *TP53* was mutated. According to the results of analysis, we identified a total of 1100 DEGs, of which 479 were raised and 621 were downregulated. The DEG volcano map is shown in Figure 6A.

GO & KEGG analysis of DEGs

We then uploaded the above 1100 DEGs that we collected and performed functional enrichment analysis through DAVID. The results of the GO analysis (Figure 6B) showed that potassium ion transmembrane transport, nervous system development, chloride transport, regulation of membrane potential, cell–cell signaling, ion transmembrane transport, transport, visual perception, regulation of ion transmembrane transport, axon guidance, cognition, cell development, regulation of DNA biosynthetic process, extracellular matrix organization and response to wounding were significantly enriched.

The KEGG pathway analysis showed a significant enrichment in neuroactive ligand–receptor interaction, cAMP signaling pathway, aldosterone synthesis and secretion, insulin secretion, calcium signaling pathway, salivary secretion, glycosphingolipid biosynthesis – lacto and neolacto series, morphine addiction, thyroid hormone synthesis and ovarian steroidogenesis (Figure 6C).

Module screening from the PPI network & the identification of prognosis related critical hub genes

In order to study the interactions of DEGs and the hub genes, we subsequently conducted research through the STRING database. Molecular complex detection helped us identify the modules in the PPI network. To find out important networks, we showed the top three significant modules (Figures 6D–F). To further find out critical genes that might contribute to the change of drug sensitivity, we did a hub gene analysis. Hub genes were selected with

Figure 3. Volcano plotting showed that cancer cells with tumor protein p53 mutation were significantly resistant to (5Z)-7-oxozeaenol, bleomycin, dabrafenib and nutlin-3a (–).

a connection degree of >10 . Then we ranked the hub genes according to this degree and the ten genes with the highest degree are regarded as the most critical hub genes, which included: *SNAP25*, *GRM5*, *SNCB*, *HRH3*, *SYT4*, *GABRG2*, *ATP2B3*, *ADCY8*, *PRKACG* and *CPLX2*. *SNAP25* had the highest degree among all the hub genes in the list with a degree of 32.

Then we analyzed whether the hub genes identified above have any relation to disease prognosis. Expression levels of the selected hub genes were divided into high or low expression levels according to the median value. Then, we used the Kaplan–Meier method with log-rank test to calculate clinical prognosis. The expression levels of *CPLX2*, *SNAP25*, *SNCB* and *ATP2B3* were significantly correlated with the disease-free survival (DFS) rate of patients (Figures 7A–D). We also found that the expression levels of *SNAP25*, *SNCB*, *SYT4*, *ATP2B3*, *CPLX2*, *GABRG2* and *HRH3* were significantly correlated with the overall survival (OS) rate of patients (Figures 7E–K). Other genes showed no influence on disease prognosis (Supplementary Figure 1). These results indicated the importance and significance of such genes in identifying and calculating disease prognosis, which might have potential research objectives in the future.

Discussion

TP53 contains multiple regulatory regions, which could complete transcriptional activation, DNA binding and other biological functions. *TP53* acts as a tumor suppressor in many types of tumors. So far, *TP53* mutations have been found to be ubiquitous in all cancer types [22]. *TP53* can respond to a variety of cellular stresses, regulate the expression of target genes, and induce cell cycle arrest, aging, apoptosis, DNA repair, or metabolic processes [23–25]. A *TP53* mutation is common in many human cancers, including glioma.

In this study, we evaluated the significance of a *TP53* mutation in drug selection and disease progression in LRGG, providing a basis for individualized treatment. We found that data from GDSC showed preliminary evidence that (5Z)-7-OZ, dabrafenib and nutlin-3a (–) exhibit resistance to LRGG cells with a *TP53* mutation. Such results may provide evidence that use of the potential drugs above, might result in ineffective medication in clinical practice.

Figure 4. Mutation of tumor protein p53 influences drug selection of lower grade glioma. (A–D) Reproduction of the GDSC database showed that lower grade glioma cells with *TP53* mutation, but not cancer of other types, was significantly resistant to (5Z)-7-oxozeaenol, dabrafenib and nutlin-3a (–), but not bleomycin.

GDSC: Genomics of drug sensitivity in cancer; IC₅₀: Half maximal inhibitory concentration; LGG: Lower grade glioma.

5Z-7-OZ is a *TAK1* inhibitor that could attenuate early brain injury after experimental subarachnoid hemorrhage and has been found to be effective in various diseases in CNS [26–28]. Dabrafenib, an inhibitor of mutated *BRAF*, has clinical activity in patients with *BRAF* V600-mutated metastatic melanoma and is a promising new therapeutic drug in treating glioma [29–32]. As for nutlin-3a (–), previous studies have recently shown that in glioma cells, activation of *TP53* by nutlin-3a decreases the expression level of $\alpha 5$ integrin to inhibit cell malignance [33]. Our findings may provide new evidence for finding better and more effective individualized treatment strategies.

In order to study mechanisms related to *TP53* mutation, we downloaded and analyzed the RNA-seq data of LRGG to identify some critical signal pathways and hub genes. The results of the GSEA analysis showed that enriched gene sets were mainly associated with *TGF- β* signaling, *NOTCH* signaling and mitotic spindles, which are all important contributors to LRGG initiation and development [3,34]. We then identified genes with differential expression and performed functional enrichment analysis of these genes. The results showed that a total of 1100 DEGs were found with different *TP53* mutation states. The differential genes of patients with *TP53*-mutated LRGG are closely related to various cellular processes. Among all of the programs, we found that programs related to ion transport were enriched significantly. As we all know, ion transport has been shown to be critical in LRGG

Figure 5. Gene set enrichment analysis results showed primary biological functional gene sets enriched in tumor protein p53 mutation in lower grade glioma patients, including *TGF- β* pathway, *NOTCH* signaling pathway and mitotic spindles.

development and treatment [35,36]. Different transportation efficiency may influence the drug transmembrane and transpiration, which might provide possible mechanisms of drug resistance.

In particular, the KEGG pathway analysis showed significant pathways that might contribute to disease progression. A previous study showed that inactivation of neuroactive ligand–receptor interaction pathway could decrease the survival of glioblastoma patients drastically [37]. LRGG with *TP53* mutation showed no significant difference with other patients, but whether the enrichment of the neuroactive ligand–receptor interaction pathway might contribute to drug resistance, could be an intriguing issue for further study. As important signaling pathways in cells, cAMP and calcium signaling pathways mediate many important signaling pathways, thus playing important roles in the progression of brain cancer [38]. These signal pathways might play important roles as targets for investigating drug resistance. In the present study, we also found that the synthesis and secretion of some metabolites and hormones, such as aldosterone, insulin, steroidogenesis and glycosphingolipid, were enriched in *TP53*-mutated LRGG patients. Although the details of them are still unclear and could involve some endocrine changes caused by the tumor, these findings provide interesting ideas for further study of the pathogenesis and drug selection for such patients.

In the PPI analysis, we found the top ten genes with the highest degree of interaction and defined them as hub genes. *SNAP25* was demonstrated to be the top one. *SNAP25* is a presynaptic plasma membrane protein regulating the release of neurotransmitters, and has been shown to have a potential biologic function in glioma [39]. We also revealed the significance of *SNAP25* in disease prognosis of DFS or OS. Our results implied the potential role of *SNAP25* and indicated that *SNAP25* may be a promising therapeutic target in glioma [40]. Our prognosis analysis results also pointed out that several other central genes, including *SNCB*, *SYT4*, *ATP2B3*, *CPLX2*, *GABRG2* and *HRH3*, are closely related to the prognosis of the disease. Among them, *CPLX2*, *SNCB* and *ATP2B3* are related to the DFS and OS rate, prompting their importance for disease progression.

Our study contained one limitation. In the present study, we only investigated the roles of *TP53* mutation in LRGG for disease prognosis and drug sensitivity and found critical pathways and hub genes. The mechanism and validation of the *TP53* mutation in this disease still needs further research in molecular biologic and clinical experiments. Moreover, it will be interesting to see how patients with *TP53* mutations respond to gold standard therapy (such as temozolomide) in terms of OS and progression-free survival, which we will investigate in future work.

Figure 6. The database for annotation, visualization and integrated discovery enrichment results of differentially expressed genes. (A) Volcano plot for differentially expressed genes. (B) The GO enrichment terms of differentially expressed genes. (C) The KEGG pathway analysis of differentially expressed genes. (D) PPI network of module top one. (E) PPI network of module top two. (F) PPI network of module top three.

GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; PPI: Protein–protein interaction.

Figure 7. The prognosis analysis of the top hub genes in lower grade glioma. (A–C) The correlation of expression levels of *SNAP25* (A), *SNCB* (B), *ATP2B3* (C) and *CPLX2* (H) with disease-free survival. Log-rank test was used for analysis and a p-value < 0.05 for statistical significance. **(D–J)** The correlation of expression levels of *SNCB* (E) and *SYT4* (F), *ATP2B3* (G), *CPLX2* (H), *SNAP25* (I), *GABRG2* (J) and *HRH3* (K) with overall survival. Log-rank test was used for analysis and a p-value < 0.05 for statistical significance.

Conclusion

In summary, our research found the novel clinical significance of a *TP53* mutation in LRGG and the associated critical genes and pathways. These results may help clinicians in identifying a better prognosis and planning more effective treatment strategies for patients.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/bmm-2020-0331

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was approved by the Research Ethics Committee of Shanghai Tenth People's Hospital, PR China and they have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

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Summary points

- A *TP53* mutant is the most frequently mutated gene in glioma, making it an important molecular biomarker for individualized therapeutic perspectives.
- The latest Cancer Genome Atlas data showed that a *TP53* mutation was present in 49% of all lower grade (World Health Organization [WHO] grades II and III) glioma patients. Data from Genomics of Drug Sensitivity in Cancer database showed that three drugs: (5Z)-7-oxozeaenol, dabrafenib and nutlin-3a (–), were more resistance to patients with a *TP53* mutation.
- A total of 1100 differentially expressed genes were identified and we did functional enrichment analysis. All the results showed that these genes are mainly concentrated in the transport of ionic and cancer-related pathways.
- The top ten hub genes were identified. The outcome analysis revealed that *CPLX2*, *SNAP25*, *SNCB* and *ATP2B3* were significantly correlated with the disease-free survival rate and that the expression levels of *SNAP25*, *SNCB*, *SYT4*, *ATP2B3*, *CPLX2*, *GABRG2* and *HRH3* were significantly correlated with the overall survival rate.
- Our results identified the key genes and pathways, which might provide some basic evidence to improve glioma individualized treatment.

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