Name: Kimin Nguyen

Analysis of TP53 mutation on LGG

Thesis:

This project has focused on the clinical, molecular, and epigenetic characteristics typical of TP53-deleterious mutations in the TCGA LGG samples. Consequently, gene expression clusters, promoter methylation status of important genes (MGMT and TERT), and IDH-specific DNA methylation patterns were compared within TP53-mutated versus non-TP53-mutated groups. In the process, we investigate other demographic and clinical variables, including tissue of origin, primary diagnosis, gender, and survival status, to place our findings into a broader perspective on how TP53 mutations would eventually affect other gene expression.

Introduction

Low-grade gliomas (LGGs) are a diverse group of primary brain tumors that frequently occur in children. While treatment outcomes for LGGs are generally favorable, tumors harboring TP53 mutations are associated with a worse prognosis compared to other mutation types or wild-type cases. This study aims to investigate how TP53 mutations influence tumor behavior by examining clinical, molecular, and epigenetic characteristics specific to deleterious TP53 mutations. Additionally, we analyze demographic and clinical variables, including tissue of origin, primary diagnosis, gender, and survival status, to contextualize these findings within a broader understanding of LGG biology and prognosis.

Methods

Sample Identification

Samples were selected from the Genomic Data Commons (GDC) database (National Cancer Institute, n.d.), specifically from The Cancer Genome Atlas Lower Grade Glioma (TCGA-LGG) dataset. The criteria for selections included Brain Lower Grade Glioma samples from patients aged 30 to 40, with open access to miRNA expression and transcriptome profiles.

Data Grouping

The dataset was divided into two groups based on TP53 mutation status. Group 2 consisted of samples with a deleterious mutation in the TP53 gene, while Group 1 included samples with no TP53 mutation. Set operations were used to separate and isolate these groups for comparison.

Data Download and Structure in R

Data was downloaded and structured in R (R Core Team, 2022) using the TCGAbiolinks package, which facilitated querying, normalization, and preparation of data in a reproducible format (Colaprico et al., 2016).

Data Filtering

Data filtering was performed using the Mutation Frequency tool on the GDC portal

(National Cancer Institute, n.d.), which was used to identify and subset samples with deleterious mutations in TP53, restricting Group 1 to clinically relevant TP53 mutations.

Differential Expression Calculation

Differential expression analysis was conducted using the DESeq2 package (Love et al., 2014) to compare expression levels between Group 1 (no TP53 mutation) and Group 2 (TP53 mutation). DESeq2 applied a negative binomial distribution model to estimate fold changes and assess statistical significance.

Result

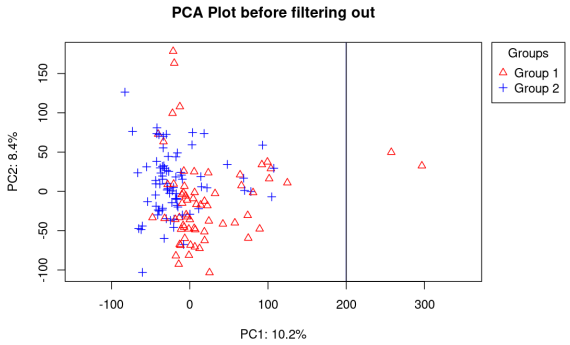

Figure 1: Prefiltering(PCA) plot. Created by ggplot 2 (Wickham, 2016) showing the distribution of samples across the first two principal components (PC1 and PC2). The X-axis (PC1) explains 10.2% of the variance, while the Y-axis (PC2) explains 8.4%, accounting for a combined 18.6% variance explained. Each point represents a sample, with different colors representing distinct groups (Group 1 as red triangles and Group 2 as blue pluses). Two outliers from Group 1 are visible on the far right which deviates from the cluster pattern

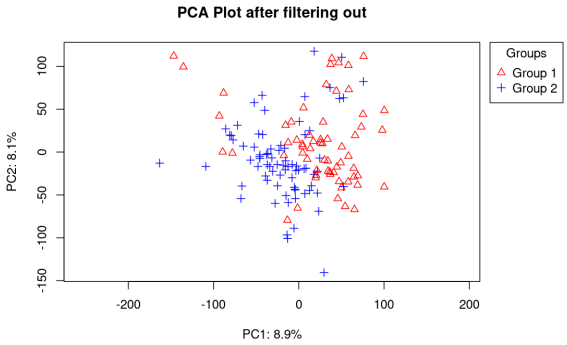

Figure 2: After filtering PCA plot. Triangles and plus signs represent Groups 1 and 2, respectively. Group 1 consists of samples without TP53 mutations, while Group 2 consists of samples with deleterious TP53 mutations. After removing two outliers identified in the previous plot, PC1 explains 8.9% of the variance, and PC2 explains 8.1%, together capturing 17% of the dataset's variability. The removal of outliers improves the clustering of the groups which provides a more accurate representation of the dataset's structure.

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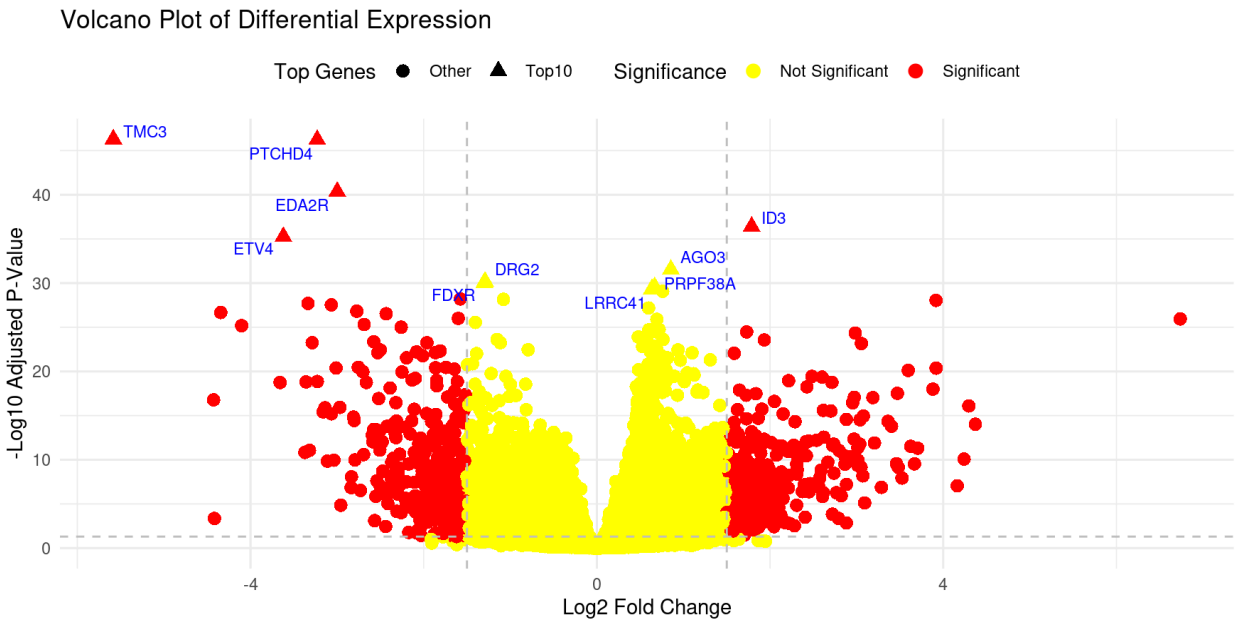

Figure 3: Volcano plot of Gene expression: After filtering, the DESeq2 analysis included a total of 43,068 genes, with 38,893 genes had non-missing adjusted p-values used for assessing the significance of differential expression. Of these, there were 5,623 genes significantly downregulated and 6,087 significantly upregulated in Group 2 (TP53 mutation) relative to Group 1 (non-TP53 mutation), which then specifically gave evidence of altered expression of genes associated with the TP53 mutation status. The 10 most significantly differentially expressed genes, ordered by smallest adjusted p-value, included TMC3, PTCHD4, EDA2R, ID3, ETV4, AGO3, DRG2, FDXR, PRPF38A, and LRRC41, emphasizing a number of potential key players in the molecular impact of TP53 mutations. From the volcano figure, most of these gene are downregulated from the TP53 mutations. The plot also appears relative symmetrical distribution~ a balance between the number of significant upregulated and downregulated genes

Analysis

TP53, known as the "guardian of the genome," encodes the tumor suppressor protein p53 that plays a critical role in maintaining genomic stability. It controls many vital functions of the cell, which include cell cycle arrest, apoptosis, DNA repair, senescence, and inhibition of angiogenesis by either repairing the damaged DNA or eliminating it through the induction of cell cycle arrest at the G1/S or G2/M checkpoints and also the induction of apoptosis with the help of pro-apoptotic genes like BAX and PUMA (*Levine, 1997*). Besides, p53 enhances DNA repair pathways such as nucleotide excision repair by activating genes like GADD45 and inhibits angiogenesis by the regulation of anti-angiogenic factors like THBS1 (*Vousden & Lane, 2007*). All these functions are disrupted by mutations in TP53, present in more than 50% of human cancers, which confer a significant oncogenic consequence (*Kandoth et al., 2013*).
Based on the differential expression between group with TP53 deleterious mutations and no TP53 mutation groups, there is a statistically supports that TMC3 was the most significantly expressed between the two groups. TMC3 (Transmembrane Channel-like Protein 3) is a member

of the TMC gene family, which encodes transmembrane proteins that are thought to function as ion channels or ion channel regulators. While TMC1 and TMC2 are well-studied for their role in mechanosensory transduction in auditory hair cells, the exact function of TMC3 remains unclear. However, its structural similarities to other TMC proteins suggest a potential role in ion homeostasis or cellular signaling pathways. TMC3 is expressed in various tissues, including neural tissues, indicating a possible role in sensory or neural processes (Keresztes et al., 2003). While there is no research regarding the regulatory relationship between TP53 and TMC genes, including TMC3.

By the gene expression analysis, TMC3 is upregulated for people without TP53 mutation and downregulated for deleterious TP53 mutations. This finding suggests that the TP53 mutations could be linked to an inhibition of TMC3 expression.

Although there is no direct evidence linking TP53 to the regulation of TMC3, it is plausible that TP53 mutations could indirectly downregulate TMC3 through the disruption of transcriptional programs, stress response pathways, or ion channel regulatory mechanisms. TP53, a tumor suppressor and transcription factor, plays a critical role in regulating genes involved in cellular stress responses, apoptosis, and ion channel activity (Levine, 1997). Mutations in TP53 often result in the loss of its regulatory function, disrupting downstream pathways and potentially decreasing TMC3 expression if it is part of p53-dependent networks. Additionally, TP53 mutations have been shown to impact genes involved in ion transport and calcium signaling, as mutant p53 alters the regulation of ion channels critical for cellular homeostasis and survival (Muller et al., 2014). Given that TMC3 belongs to the TMC family, which regulates ion channels, its downregulation may be part of a broader effect on ion channel pathways disrupted by TP53 mutations. Furthermore, certain gain-of-function TP53 mutations actively repress tumor-suppressive genes or enhance oncogenic signaling pathways, which may further suppress TMC3 expression or activity if it plays a role in growth inhibition (Brosh & Rotter, 2009). These mutations also disrupt cellular stress responses, and if TMC3 participates in these pathways, its downregulation could be a consequence of TP53 dysfunction (Vousden & Lane, 2007). While direct evidence is lacking, these findings suggest that the impact of TP53 mutations on TMC3 warrants further investigation, particularly in the context of ion channel regulation and cancer progression.

Discussion

These results have broader implications that suggest the possibility of TP53 mutations having more pervasive impacts on cellular processes than previously appreciated and that may extend to ion channel activities and cellular signaling networks. Ion channels play a very important role in cellular stress responses, signaling, and ion homeostasis-functions critical for the maintenance of normal cell physiology and the prevention of tumorigenesis. Disruptions in these processes, as might occur through TP53 dysfunction, could provide insights into new mechanisms by which cancer cells evade normal regulatory controls((Muller et al., 2014). However, the study's sample size is limited, with 63 samples in the control group and 77 in the TP53 mutation group, which may affect the generalizability of the findings. Additionally, the molecular mechanisms linking TP53 and TMC3 are not fully understood. While statistical analyses indicate that TP53 mutations are associated with TMC3 downregulation, further experimental validation is necessary.

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Although a direct link between TP53 and TMC3 has not been established, observed expression patterns warrant investigation into indirect mechanisms. Previous studies have shown that TP53 mutations can disrupt transcriptional programs and stress response pathways, potentially affecting genes like TMC3 (Muller et al., 2014). Moreover, TP53 mutations have been reported to alter the regulation of genes involved in calcium signaling and ion transport, functions closely related to TMC proteins (Kurima et al., 2015). These broader regulatory disruptions may underlie the downregulation of TMC3 in the presence of mutant TP53, necessitating further mechanistic studies.

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