Comprehensive Analysis of TP53 Mutation Characteristics and Identification of Patients with Inferior Prognosis and Enhanced Immune Escape in Diffuse Large B Cell Lymphoma

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Research

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Abstract

Background:

Diffuse large B-cell lymphoma (DLBCL) is a highly heterogeneous lymphatic malignancy. The role of *TP53* gene alterations in DLBCL remains unclear.

Methods:

We performed a comprehensive analysis of the genomic characteristics of *TP53* through targeted next-generation sequencing (n=176), RNA-sequencing (n=152), and circulating tumor DNA sequencing (n=38).

Results:

TP53 was frequently mutated in DLBCL; most *TP53* mutations occurred in the DNA-binding domain (DBD). However, *TP53* alone is insufficient to effectively differentiate the risk of DLBCL, even when only considering mutations in the DBD region. However, CD58 mutations, which are mutually exclusive from *TP53* mutations, in combination with *TP53* mutations, could significantly differentiate the prognosis of DLBCL. The survival of patients with either one of the mutually exclusive mutation patterns, namely, TP53MUT&CD58WT or TP53WT&CD58MUT, was inferior to those harboring both wild-type *TP53* and CD58. Notably, patients with the TP53WT&CD58MUT mutation pattern had the worst outcome and were characterized by an enhanced immune escape, including features such as the abundant infiltration of inflammatory cells and upregulation of inhibitory immunomodulatory molecules; these patients represent the candidate populations for immune therapy.

Conclusions:

Our findings indicated that the mutation patterns of *TP53* and CD58 accurately stratified patients with DLBCL to permit the optional immunotherapy.

Background

Diffuse large B-cell lymphoma (DLBCL), the most common and one of the most aggressive types of non-Hodgkin lymphoma, is highly heterogeneous from both the clinical and molecular standpoints. Despite the possibility of cure through immunochemotherapy, primary refractor or relapse occurs in approximately 40% of patients, with dismal outcomes. Next-generation sequencing technologies have enabled a more comprehensive description of the molecular heterogeneity of DLBCL, which may aid in risk stratification and the design of alternative management strategies.

TP53, a well-known tumor suppressor gene, plays a crucial role in cell cycle arrest, DNA repair regulation, autophagy, and apoptosis. *TP53* mutations have been observed in DLBCL, with a mean frequency of 20%. Studies on *TP53* mutations as prognostic markers have historically been controversial, and the results have not been consistent across different studies on DLBCL. Considering the complex

pathophysiological mechanisms involved in DLBCL, we wondered whether the interaction of *TP53* with other genetic variants could further promote the development of DLBCL, and thus be more prognostically predictive. Moreover, whether the genetic interactions between *TP53* and other oncogenic mutations could shape the discrepant immune landscape in DLBCL remains unknown, as these genetic alterations usually drive the malignant phenotype and directly or indirectly affect the tumor microenvironment (TME) and support tumor survival.

Given that the DNA-binding domain (DBD) of *TP53* is the functional central core domain and mutations in this region potentially have a strong impact on TP53 function, in the current study, we performed a comprehensive analysis of *TP53* mutations in the DBD region. Genome alterations that were mutually exclusive or co-occurring with *TP53* mutations were identified, and their potential value in prognosis and possible relationships with TME were further examined using targeted next-generation sequencing and transcriptome sequencing.

Materials And Methods

Patients and samples

Frozen tumor biopsy specimens were obtained at diagnosis from patients with *de novo* DLBCL at Tianjin Medical University Cancer Institute and Hospital, among which, sufficient quantity and quality genomic DNA (n = 176) and relevant RNA (n = 152) from tumor biopsies were available for high-throughput sequencing. Another cohort of 38 DLBCL patients was enrolled, and dynamic plasma samples were collected for circulating tumor DNA (ctDNA) testing. All biopsies were reviewed independently by two experienced pathologists for diagnostic confirmation. The mutation data of 1,001 DLBCL patients were downloaded from cBioPortal (<http://www.cbioportal.org/>).

Targeted next-generation sequencing

DNA was extracted using the DNeasy Tissue and Blood Kit (Qiagen, Venlo, Netherlands). Targeted exome capture and next-generation sequencing was performed on a customized 307-gene panel using the SureDesign Tool (Agilent Technologies, CA, USA) (**Supplementary Table S1**). Subsequently, libraries were prepared using the Agilent SureSelectXT 0.5-2.9Mb Custom kit (Agilent Technologies) and sequenced on an Illumina sequencing platform, and 150 bp paired-end reads were generated. Sequencing data is available from the CNGB Sequence Archive of China National GeneBank DataBase under the accession number, CNP0001322.

Identification of somatic single nucleotide variations and indels

Valid sequencing data were mapped to the reference human genome (UCSC hg19) using the Burrows-Wheeler Aligner software. Substitutions and indels were identified using GATK HaplotypeCaller and then

filtered to remove potential contamination from germline polymorphisms following the criteria described in our previous study.

Somatic mutation analysis

Fisher's exact test was used to identify genes that showed a strong mutual exclusivity or co-occurring pattern with *TP53* mutations. Tumor mutational burden (TMB) was assessed based on the total number of deletions, insertions, base substitutions, or somatic gene coding errors detected per million.

Detection of *TP53* alterations in ctDNA

A 31-gene panel was designed for ctDNA sequencing, including 17 *TP53* mutation sites, eight common hotspot mutations, and nine other mutations frequently detected in tumor samples (**Supplementary Table S2**). Circulating single-molecule amplification and resequencing technology was used to detect dynamic alterations of *TP53* in serial ctDNA samples.

RNA-seq and gene expression analysis

RNA was extracted using an RNeasy Kit (Qiagen, Hilden, Germany). Sequencing libraries were generated using the NEBNext® UltraTM RNA Library Prep Kit for Illumina and 150 bp paired-end reads were generated. RNA-seq data is available from the CNGB Sequence Archive of China National GeneBank DataBase under the accession number, CNP0001327.

Reads were aligned to the human reference genome and transcriptome hg38. The expression values of each gene were normalized using fragments per kilobase per million. Differentially expressed genes were identified using the R package, DESeq2.

Functional enrichment analysis was performed using the clusterProfiler package (gene ontology analysis) and Java desktop software (gene set enrichment analysis).

Immune cell infiltration analysis

ESTIMATE was used to estimate scores representative of the relative proportion of tumor purity and stromal and immune cells. Immune cell abundance identifier, a gene set signature-based method, was used to estimate the abundance of 24 immune cells.

Statistical analyses

Mann-Whitney U test, the chi-square test, or Fisher's exact test were used for comparisons between groups, as appropriate. Overall survival (OS) was defined as the time from diagnosis to death due to any cause. The Kaplan-Meier log rank test was used for survival comparison. Statistical significance was set at $p < 0.05$.

Results

Genetic landscape of DLBCL

Targeted next-generation sequencing was performed in 176 patients with *de novo* DLBCL. The mean sequencing depth was 860×, and a mean of 99.0% of the target sequence was covered. A total of 5,117 non-silent mutations, including 4,299 missense, 475 indel, 232 nonsense, 109 splice site, and 2 nonstop mutations, were identified. The most frequently mutated genes are listed in Fig. 1A. *TP53* was the third most frequently mutated gene, with a rate of 30% (53 of 176). We proceeded to compare the median variant allele frequencies of targeted genes. Mutations in genes, such as *TRIM56*, *TMEM184A*, and *TLN2* occur early in DLBCL pathogenesis, and *HIST1H2AM*, *SPATA31C1*, and *MST1L*, are late events (Fig. 1B). The occurrence of *TP53* mutation was skewed toward the early stages of DLBCL pathogenesis.

The *TP53* gene is mainly targeted by missense mutations in the DBD region in DLBCL

We identified 62 sequence variants of *TP53* in 53 DLBCL biopsies. Among these variants, 74% (n = 46/62) were missense mutations, and the remaining were inactivating frameshift indels (n = 7), nonsense mutations (n = 3), coding sequencing indels (n = 4) and splicing mutations (n = 2). Mutation patterns and distributions are shown in Fig. 2A, B, and E, and **Supplementary Table S3**. Importantly, most mutations (56/64, 87.5%) occurred in exons 5–8, which encoded the DBD region of *TP53* (Fig. 2C). Codons 175, 273, and 248 of the p53 protein had the highest mutation frequency, which are also the hot spots of *TP53* mutation found in most human cancers (Fig. 2D). Given the important role of the DBD of *TP53*, we mainly focused on mutations in this region in the following analysis.

Prognostic value of *TP53* mutation status in DLBCL

The clinical features of patients with and without *TP53* mutations (*TP53*-MUT and *TP53*-WT) in the DBD region are shown in **Supplementary Table S4**. The age, sex, B symptoms, stage, number of extranodal sites, performance status, LDH level, and International Prognostic Index distribution were similar between the two groups. Among the 176 patients, 155 patients having the complete follow-up data after receiving the front-line treatment were enrolled in the analysis between *TP53* mutation status and clinical outcomes. Overall, patients with *TP53*-MUT tended to have inferior OS compared to patients with *TP53*-WT (median: 92.3 versus 110.8 months, respectively, $p = 0.168$), but not to a level of statistical significance (Fig. 2F). However, we found that patients with *TP53* mutations in blood-derived ctDNA were significantly associated with poorer OS than patients with negative *TP53* alterations in ctDNA (Fig. 2G). Moreover, it seems that monitoring *TP53* changes could predict therapeutic responses in DLBCL (**Supplementary Fig. 1**).

TP53 mutation status and genomic instability

TP53 has been called the “guardian of the genome”. We further assessed whether *TP53* alterations were responsible for genomic instability in DLBCL at the individual nucleotide level. Nevertheless, there were no differences in the number of deletion, insertion and SNV events between the *TP53*-MUT and *TP53*-WT groups. Moreover, the TMB was similar between the two groups (Fig. 3A–E).

Genomic interactions correlated with TP53 mutation status distinguish the prognosis of DLBCL patients

To catalog the alterations that share similar oncogenic endpoints with *TP53* mutations, we recognized the genomic variants that co-occur or are mutually exclusive with *TP53*. We observed that *DDX3X*, *MYLK2*, and *FUT6* mutations co-occurred with *TP53* mutations, and *CD58* mutations were mutually exclusive with *TP53* mutations (Fig. 4A and **Supplementary Table S5**), which led us to create a new risk stratification based on these features. Specifically, patients were divided into four groups based on the mutation status of these genes. No significant difference was observed in survival among the four groups according to the combination of co-occurring mutation genes with *TP53*. However, we found that the combination of *TP53* and *CD58* mutations could significantly distinguish the prognosis of patients with DLBCL (Fig. 4B–E). Patients with both wild-type *TP53* and *CD58* had a better prognosis than patients with either of the two mutually exclusive modes of *CD58* and *TP53* mutations. Unexpectedly, patients with *TP53* wild-type and *CD58* mutations (TP53WT&CD58MUT) had worse survival than those with *TP53* mutations and *CD58* wild-type (TP53MUT&CD58WT). Because *TP53* and *CD58* mutations were mutually exclusive, only one patient harbored both *TP53* and *CD58* mutations, and the patient still alive at the last follow-up. Moreover, the relationship of a mutually exclusive mutant between *CD58* and *TP53* and the prognostic significance of this interaction was validated using publicly available data from 1,001 patients with DLBCL (**Supplementary Fig. 2**).

Mutually exclusive patterns between TP53 and CD58 mutations confer distinct immune infiltration

Given the potential role of *CD58* in the recognition of tumor cells by immune surveillance mechanisms, we wondered whether the cooperation of the mutually exclusive mutations between *TP53* and *CD58* may further profoundly influence the microenvironment in DLBCL. We first assessed whether there was a difference in TMB between the TP53MUT&CD58WT group and TP53WT&CD58MUT group, as TMB may have a potential role in shaping TME due to increased neoantigen exposure. Interestingly, we found that the overall TMB was significantly higher in the TP53WT&CD58MUT group than in the TP53MUT&CD58WT group (Fig. 5A), while there was no difference in TMB when dividing patients only according to *TP53* mutation status (Fig. 3B). Next, we applied the ESTIMATE algorithm to estimate the relative fraction of stromal and immune cells and tumor purity in DLBCL TME. We found that the ESTIMATE immune score in the TP53WT&CD58MUT group was higher than that in the TP53MUT&CD58WT groups, and the tumor purity and stromal score did not differ between the two groups (Fig. 5B–D).

We then estimated the abundance of infiltrating immune cells between the two groups. We found that the levels of exhausted T cell, macrophage cell, NK cell, and Th1 cell infiltration were significantly higher in the TP53WT&CD58MUT group than in the TP53MUT&CD58WT group (Fig. 5E). To determine whether this difference was only affected by the *CD58* mutations or the result of the combined action of *CD58* and *TP53*, we compared the immune infiltration cells between patients with and without *CD58* mutations. We found that the immune cell infiltration was similar between the *CD58* wild-type and mutation groups, except for CD8 naïve T cells (**Supplementary Fig. 3**). This result suggests that the differences in the

infiltrating immune cells between the two groups were due to the combined influence of the mutation pattern 'TP53WT&CD58MUT'.

Mutually exclusive patterns between TP53 and CD58 mutations link to distinct immunomodulatory molecule expression

The findings that infiltrating immune cells enriched in the TP53WT&CD58MUT group prompted us to examine the expression of immunomodulatory molecules on these immune cells. An initial analysis highlighted distinct expression patterns of immunomodulators in the TP53WT&CD58MUT group in comparison with the TP53MUT&CD58WT group (Fig. 5F **and Supplementary Table S6**). The co-inhibitory receptors, such as PD-1, TIM3, and LAG3 on T cells, KLRC1, KLRC3, and KLRD1 on NK cells and the inhibitory enzymes IDO1, IDO2, and TDO2 were preferentially expressed in the former group (Fig. 5G). However, there was no difference in the expression of co-stimulatory molecules between the two groups (Fig. 5F).

In addition, we found that inhibitory immunomodulators were also significantly upregulated in the TP53WT&CD58MUT group when comparing with the TP53WT&CD58WT group, which suggested the unique immune phenotype in the TP53WT&CD58MUT group (**Supplementary Fig. 4**). Overall, the observations that enriched immune cell infiltration and frequent inhibitory immunomodulator expression strongly indicate the enhanced immune escape in the TP53WT&CD58MUT group.

Mutually exclusive patterns between TP53 and CD58 mutations harbored distinct immune microenvironment

Functional enrichment analysis was conducted to elucidate the differentially biological pathways between the TP53WT&CD58MUT group and the TP53MUT&CD58WT group. Five hundreds differentially expressed genes were identified with a false discovery rate less than 0.05 and $|\log_2\text{foldchange}| > 1$ (Fig. 6A). One hundred genes were significantly upregulated and 400 genes were significantly downregulated in the TP53WT&CD58MUT group compared with the TP53MUT&CD58WT group. Gene ontology analysis showed that a number of terms related to cytokine and chemokine and inflammatory pathways were enriched in the TP53WT&CD58MUT group, including cytokine and chemokine production, binding and activity, and interferon- γ pathway (Fig. 6B). We also found that IDO and TDO activities were upregulated in the TP53WT&CD58MUT group, concordant with the high expression of IDO-1, IDO-2, and TDO-2 in this group (Fig. 6B). Additionally, GSEA demonstrated that interferon- α and - γ responses and IL-6/JAK/STAT3 signaling enriched in the TP53WT&CD58MUT group (Fig. 6C). Together, our findings that a lot of immune related biological pathways enriched in the TP53WT&CD58MUT group indicated a relatively abundant inflammatory immune microenvironment. Nevertheless, abundant infiltrating exhausted T cells and multiple inhibitory immunomodulators were elevated in this group, which finally lead to the immune escape of DLBCL cells and inferior prognosis for patients.

Discussion

The value of *TP53* gene alterations in predicting survival in DLBCL remains controversial, even in the era of sequencing. A previous study identified a molecular subgroup (C2) characterized by inactivation of *TP53* and showed that patients with this subgroup had a trend of progression over time. Nevertheless, a subsequent study demonstrated that the impact of *TP53* mutations on survival was relied on the genetic context of the lymphoma, conferring no effect in the SOCS1/SGK1 clusters and NOTCH2 subtype and inferior prognosis in the MYD88 subtype. The study by Young and colleagues suggested that *TP53* mutations in the DBD were the strongest predictor of poor OS. However, in our study, a tendency toward inferior survival was observed in patients with *TP53* DBD mutations, but it was not statistically significant. It appears that *TP53* alone is insufficient to effectively differentiate the risk of DLBCL, as clinical decision-making is becoming increasingly based on individual genomic data. Given the small number of patients that could be accurately classified according to the above molecular classification, we could not confirm the findings regarding *TP53* in our study.

The interaction mode of genetic mutations, whether co-occurring or mutually exclusive mutations, drive tumor initiation and progression consistently by cooperating pathways or the same pathway. We found that only *CD58* mutations in combination with *TP53*, in a mutually exclusive manner, could significantly differentiate the prognosis of DLBCL. Patients with mutually exclusive mutation pattern, both *TP53*WT&*CD58*MUT and *TP53*MUT&*CD58*WT, experienced inferior survival compared to patients with both wild-type pattern. In particular, patients with *TP53*WT&*CD58*MUT had even worse outcomes than those with *TP53*MUT&*CD58*WT.

Interestingly, we found that patients with *TP53*WT&*CD58*MUT harboured enhanced immune escape capacity, giving the abundant infiltration of exhausted T cells and multiple upregulated inhibitory immunomodulatory molecules in this group. It appears that escape from elimination by immune effector cells is an important contributor to the development of DLBCL. Despite many of such immune dysregulation had an adverse prognosis; they provided new opportunities for anti-tumor immunotherapy of the subset of DLBCL patients with *TP53*WT&*CD58*MUT. Historically, the response rate to anti-PD-1/PD-L1 therapy in unselected DLBCL patients was generally low. It highlights the importance of identifying novel therapeutic vulnerabilities and reasonable combination therapy strategies, such as different immune checkpoint inhibitors or in combination with tryptophan dioxygenase (IDO1/TDO2) inhibitors. Consequently, patients with *TP53*WT&*CD58*MUT may be optimal candidates for novel immunotherapy in clinical trials. In addition, a tailored treatment needed to be further explored for patients with other subtypes.

Conclusion

In conclusion, our results suggest that *TP53* mutation alone is insufficient to effectively differentiate the risk of DLBCL. The mutually exclusive patterns between *TP53* and *CD58* mutations will contribute greatly

towards the formulation of strategies for the efficient diagnosis and management of DLBCL and will aid decision making with regard to the choice of appropriate treatment methods for DLBCL patients.

Abbreviations

ctDNA: circulating tumor DNA

DBD: DNA-binding domain

DLBCL: diffuse large B-cell lymphoma

TME: tumor microenvironment

TMB: tumor mutational burden

OS: overall survival

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of the Tianjin Medical University Cancer Institute. Informed consent was obtained from all patients.

Consent for publication

Not applicable

Conflict of interest

The authors declare no conflict of interest.

Data availability

DNA and RNA sequencing data have been submitted to the CNGB Sequence Archive of China National GeneBank DataBase (<https://db.cngb.org/cnsa/>) under the accession number CNP0001322 and CNP0001327, respectively. The published mutation data of 1,001 DLBCL

patients is available from cBioPortal (<http://www.cbioportal.org/>).

Authors' contributions

X.W. conceived and designed the study; X.W. and H.Z. supervised all aspects of research project and interpreted data; T.Z., Y.L. and X.L. performed the research, statistical and bioinformatics analyses; M.Z.

performed the next-generation sequencing and bioinformatics analysis; J.H., X.L., L.L., L.Q., Z.Q. and S.Z. collected samples and clinical information; B.M. and Q.Z. reviewed the diagnosis of DLBCL; X.W., H.Z. and X.R. provided the clinical samples and material support; T.Z. wrote the manuscript and finalized the figures; X.W. and H.Z. reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Figures

Figure 1

Figure 1

Genomic alterations in diffuse large B cell lymphoma (DLBCL). A. Spectrum of the Top 30 genetic alterations in DLBCL. Samples are displayed in columns. The overall number of somatic mutations per patient and per gene are shown at the top and on the right, respectively. Different colors represent different types of alteration. B. Proposed temporal order of recurrently mutated genes in DLBCL based on their median variant allele frequency (VAF); the distribution of the VAF for each gene across the entire cohort is displayed as a boxplot. Higher medians indicate higher VAF and therefore may correspond to earlier events in tumorigenesis.

Figure 2

mutation type; TP53-WT:TP53 wild-type. The Kaplan–Meier method and log-rank test were used for survival analyses.

Figure 3

Figure 3

TP53 mutation status and genomic instability at the individual nucleotide level. A. The distribution of deletion, insertion, single nucleotide variant (SNV), and tumor mutation burden (TMB) in patients with TP53-MUT and TP53-WT. The colors represent the type of alteration. B-E. Comparison of the number of deletions, insertions, SNVs, and TMB in patients with TP53-MUT and TP53-WT, respectively. There was no significant difference observed between the two groups regarding these variables. $p < 0.05$ is significant.

Figure 4

Figure 5

Figure 5

Comparison of immune-associated characteristics between the two groups with distinct mutually exclusive mutation patterns of TP53 and CD58. A. TMB in the TP53MUT&CD58WT group versus the TP53WT&CD58MUT group. B. Immune scores, (C) tumor purity, and (D) stromal scores in the TP53MUT&CD58WT group versus the TP53WT&CD58MUT group. E. Immune infiltrating cells in the TP53MUT&CD58WT group versus the TP53WT&CD58MUT group. F. Immunomodulatory molecule

expression in the TP53MUT&CD58WT group versus the TP53WT&CD58MUT group. G. Significantly up-regulated immunomodulatory molecules in the TP53WT&CD58MUT group versus the TP53MUT&CD58WT group. *, $p<0.05$; **, $p<0.01$; NS, $p>0.05$.

Figure 6

Figure 6

RNA-seq identifies differentially expressed genes in the TP53WT&CD58MUT group relative to those in the TP53MUT&CD58WT group. A. Heatmap depicting 200 differentially expressed genes in DLBCL patients with TP53MUT&CD58WT versus those with TP53WT&CD58MUT (FDR-adjusted p -values <0.05 , $|\log_2\text{foldchange}|>1$). B. Gene ontology analysis performed on the significantly upregulated genes in the TP53WT&CD58MUT group. Selected and significantly enriched (FDR-adjusted p -value <0.05) gene ontology annotations for biological processes and molecular functions are represented as dots. C. GSEA demonstrating enrichment of interferon- α and - γ responses and IL-6/JAK/STAT3 signaling in the TP53WT&CD58MUT group compared with the TP53MUT&CD58WT group ($|\text{normalized enrichment score}|>1$, NOM p -val <0.05 , FDR q -val <0.25). FDR, false discovery rate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalFigures.pdf](#)
- [SupplementalTables.docx](#)