# Features and therapeutic potential of T-cell receptors in high-grade glioma

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## Abstract

**Background:** Previous studies have shown that endogenous T cells play an important role in the prolonged survival time of high-grade glioma (HGG) patients. Our objectives were to investigate the features of T-cell receptor (TCR) repertoires in HGG patients and to elucidate any potential therapeutic value.

**Methods:** During November 2011 and December 2018, tumor tissues and blood samples of 35 patients with HGG who underwent surgery at Beijing Tiantan Hospital or Beijing Shijitan Hospital were selected after surgery. After isolating DNA from samples, multiple rounds of PCR were performed to establish a DNA immune repertoire (IR). Then, the sequences and frequencies of the complementarity-determining 3 (CDR3) region in TCR beta chain (TRB) were identified by high-throughput sequencing and IR analysis. A survival follow-up was conducted monthly thereafter until December 2018. Finally, the *t* test and Mann-Whitney test were used to compare statistical differences between two sets of data.

**Results:** The Shannon diversity index (SHDI) of TRB sequences of HGG patients was significantly lower than that of healthy individuals (7.34 *vs.* 8.45, $P = 0.001$). The SHDI of TRB sequences of glioblastoma (GBM) patients with more than 16 months survival time was much higher than that of GBM patients with shorter survival times in both tumor tissues ($3.48 \pm 0.31$ *vs.* $6.21 \pm 0.33$, $t = -5.49$, $P = 0.002$) and blood cells ($6.02 \pm 0.66$ *vs.* $7.44 \pm 0.32$, $t = -2.20$, $P = 0.036$). In addition, patients achieved a distinctly higher proportion compared to that of healthy individuals in the proportion of TRBV9 and TRBV5 functional regions (9.83% *vs.* 6.83%, $P = 0.001$). Surgical tissue from patients who survived more than 16 months yielded a much higher proportion of TRBV4 and TRBV9 regions (7.14% *vs.* 3.28%, $t = 3.18$, $P = 0.019$). In surgical tissues from two GBM patients who survived for longer than 46 months, we found a potentially therapeutic TCR sequence.

**Conclusions:** HGG patients have less species diversity of TCR repertoires compared with that of healthy individuals. TRBV9 regions in TCRs may be protective factors for long-term survival of GBM patients.

**Keywords:** Glioma; T-cell receptor; Immunotherapy; Survival; Therapeutics

## Introduction

High-grade glioma (HGG) is a major primary malignant tumor of the central nervous system, with a high recurrence rate and extremely high mortality worldwide.[1-3] Among the types of HGG, the most malignant type is glioblastoma (GBM). The average survival time of GBM patients was only 14.6 months, and their 5-year survival rate was only 5.5%.[3] Currently, the standard treatments are surgery, post-operative radiotherapy and chemotherapy.[4,5] Therefore, searching for new therapies that can prolong the lifetime of patients is urgently needed.

Recently, studies have found that T-cell immunotherapy has become one of the most promising treatments for various tumors in terms of prolonging survival time and improving living quality.[6-10] Among these studies, a striking clinical trial indicated that endogenous T cells play a significant role in the prolonged survival time of HGG patients.[10] On the surface of T cells, there are T-cell receptors (TCRs) that can specifically recognize and bind to antigens. TCRs play an important role in inducing apoptosis.[11-14] Additionally, cloned TCRs have been recommended by the Cancer Research Center from National Institutes of Health (NIH) as the main factor that causes tumor reduction or disappearance in patients.[15] In fact, TCR-engineered T-cell (TCR-T) immunotherapy is based on the application of artificially modified TCRs and is especially suitable for solid tumors.[16-20] Therefore, the study on TCRs is an important way to find potential therapeutic targets for various tumors. Studies have shown that 95% of mature TCRs are connected

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by alpha chains and beta chains through disulfide bonds. Beta chains are much more variable than alpha chains. Additionally, in TCRs' beta chains (TRBs), the third complementarity-determining region (CDR3) is unique to each T-cell clone because it can bind directly to the antigen.[21-25]

The purposes of the present study were to investigate the features of TCRs in both tissue and venous blood of HGG patients, and to find novel-specific TRB sequences from patients with longer survival times that can identify glioma cells with potential clinical therapeutic value. Both the purposes have been rarely reported previously.[26]

## Methods

### Ethical approval

The research was conducted in accordance with the *Declaration of Helsinki* and was approved by the local ethical committee of Beijing Shijitan Hospital (2017 Research Ethics Review No. [7]). All patients provided informed consent.

### Sample collection and DNA isolation

During November 2011 and July 2018, 35 HGG patients' venous-blood samples after resection with all clinical pathological parameters — as well as paired tumor tissue samples from ten patients — were obtained from Beijing Tiantan Hospital or Beijing Shijitan Hospital. Before surgery and within 20 days prior to blood collection, none of the patients received radiotherapy or chemotherapy. The sample contents are summarized in Supplementary Table 1, http://links.lww.com/CM9/A46. Additionally, 101 cases of venous blood-cell specimens from healthy individuals, aged 19 to 65 years old, were collected by the clinical laboratory staff of Geneplus-Beijing Institute (China).

First, 3 mL of venous blood per person was collected using an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. Second, DNA from tumor tissues was extracted using a commercial kit (Maxwell® 16 FFPE Plus LEV DNA Purification, Qiagen, Hilden, Germany, Kit catalog: AS1135). In addition, DNA from blood lymphocytes was isolated by another commercial kit (QIAamp DNA Mini Kit, Qiagen, Catalog: AS1135).

### TRB sequencing and sequence screening

CDR3 of TRB was implemented by multiple rounds of PCR, inclusively and semi-quantitatively, to establish a DNA immune repertoire (IR).[27,28] Raw sequencing data for TRB was processed and analyzed via the following sequence: (1) filtering of raw reads to remove the undesired sequences that did not contain the primers for multi-PCR using cutadapt[27] (https://github.com/marcelm/cutadapt); (2) merging the remaining high-quality paired reads to obtain contigs using Pear[28] (https://pear.php.net/copyright.php); and (3) spotting of the CDR3 region and the TRB V/(D)/J gene segment using MiXCR IR analysis software (https://github.com/milaboratory/mixcr/).[29]

According to previous experiments, we empirically determined that the TCR sequences with a clone frequency of less than $10^{-5}$ were caused by systematic errors.[27] Hence, our subsequent studies focused on TCR sequences with a clone frequency of $\geq 10^{-5}$.

The diversity, clonality, and frequency of TCRs were obtained to characterize the features of IRs. The diversity of the TCR sequences was calculated based on the Shannon-Wiener diversity index (SHDI). A fixed number of TCRs was defined as 80% of the smallest sample, and we randomly subsampled that number of TCRs from each sample. Then, SHDI was calculated as follows. In SHDI, ni is the number of copies of a specific clonotype, $S$ is the number of different clonotypes, and $N$ is the total number of TRB sequences analyzed.

$$\text{Shannon index} = -\sum_{i=1}^{S} \frac{\text{ni}}{N} \ln \frac{\text{ni}}{N}$$

### Follow-up

The time of HGG resection was taken as the starting time of post-operative survival. Patients were then treated with radiotherapy and chemotherapy and were followed up with a combination of telephone conversations and monthly surveys. The overall survival (OS) was obtained in the content of the follow-up appointments. The last follow-up time was December 2018.

### Statistical analysis

Seventy cases were randomly selected from the 101 healthy individuals. Then, their data were used as healthy control data and were compared with data from 35 HGG patients. The results are expressed as mean ± standard error or median (range), as appropriate. Comparisons of proportions and variables between two different groups were performed using IBM SPSS Statistics 20 statistical software (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test was performed for normality, and then the normal distribution data were further tested for homogeneity of variance. Afterwards, two-independent-sample $t$ test was used for data with equal variance, and two-independent-sample $t'$ test was used for data with variance unequal. The non-normal distribution data was compared by Mann-Whitney $U$ test. Using a two-sided analysis, $P \leq 0.05$ was considered statistically significant.

Perl language from Active Perl_5.16 for Windows (Microsoft Corporation, USA) was adopted to code for excluding the TCRs that could bind to the virus. The TCR sequences with therapeutic potential that were expressed simultaneously in the tissue of at least two cases were searched.

## Results

### High-throughput sequencing of TRB repertoires

In each healthy individual's blood, the total reads of TRB sequences was 142792–28614001, and the diversity was 2607–23084. Meanwhile, in every HGG patient's blood sample, the total reads of TRB sequences were

190766–8302256, and the reads of unique TRB sequences were 152–31797. Moreover, in tissue samples of HGG patients, the total reads of TRB sequences was 97948–7964839, and the diversity was 82–15704.

### Follow-up of patients with HGG

During January 2017 and December 2018, the follow-up data showed that two HGG patients were withdrawn; the OSs of 33 patients with HGG are shown in Supplementary Table 1, http://links.lww.com/CM9/A46.

### Difference of SHDI in TRB sequences between healthy individuals and HGG patients

The Kolmogorov-Smirnov test showed that the SHDIs in TRB sequences of healthy individuals was non-normal distribution data ($P = 0.026$). The Mann-Whitney test showed that, in blood cells, the SHDI of TRB sequences in 35 HGG patients was significantly lower than that of healthy individuals ($P = 0.001$) [Figure 1]. The median SHDI in HGG patients was 7.34, while in healthy individuals, it was 8.45. HGG patients exhibited less diversity of TCRs than that of healthy individuals.

### Difference of SHDI in TRB sequences between long-term and short-term survival HGG patients

The Kolmogorov-Smirnov test showed that the SHDIs in TRB sequences of HGG patients were normal distribution data. As shown in Figure 2, after analysis with the two-independent-sample $t$ test, we found that the SHDI of TCRs in blood cells in HGG patients with <16 months survival time (short-term survival, STS) was prominently lower than that of HGG patients with longer survival times (long-term survival, LTS; mean value: $6.02 \pm 0.66$ *vs.* $7.44 \pm 0.32$; $t = -2.20$; $P = 0.036$). Additionally, in tumor tissues, GBM patients with <16 months survival time showed an even lower SHDI than that of GBM patients with longer survival times (mean value: $3.48 \pm 0.31$ *vs.* $6.21 \pm 0.33$; $t = -5.49$; $P = 0.002$).

Considering functional regions of TCRs, in blood, HGG patients achieved a distinctly higher level than that of healthy individuals in terms of the proportions of TCRs with the following functional regions. As shown in [Figure 3], the proportions of TCR in TRBJ2-1 [Figure 3A] and TRBJ2-7 [Figure 3B] showed, respectively, significant differences (median value: 12.95% *vs.* 9.85%, $P = 0.001$; median value: 31.94% *vs.* 25.22%, $P = 0.001$). In addition, the comparison of HGG patients and healthy individuals in TRBV4 and TRBV9 + TRBV5 regions is shown in Figure 3C and 3D. The above proportion of blood TCR in HGG patients was much higher than in healthy individuals (median values: 2.72% *vs.* 2.11%, $P = 0.017$; 9.83% *vs.* 6.83%, $P = 0.001$).

In surgical tissues, as shown in Figure 4, patients who survived for <16 months obtained a much lower proportion of TRBV9 + TRBV4 regions (mean value: $3.28 \pm 0.70\%$ *vs.* $7.14 \pm 0.99\%$; $t = -3.18$; $P = 0.019$) [Figure 4A]. In addition, between STS and LTS patients, the sum proportion of TRBJ2-2 and TRBJ2-7 also varied remarkably (mean value: $20.02 \pm 2.42\%$ *vs.* $39.74 \pm 4.39\%$; $t' = -3.23$; $P = 0.018$) [Figure 4B].

In surgical tissues of two GBM patients (patient No. 16 and patient No. 11) who survived longer than 46 months, we found the same amino acid sequence from TRB. This TRB sequence had functional regions of both TRBJ2-1 and TRBV9. In the tissue sample of patient No. 16, the fractional number of this amino acid sequence out of the total amino acid sequences from TRB was 3.37%. Additionally, through high-throughput sequencing, we discovered that the sequence of these amino acids was CASSVTSGRSNEQFF. This sequence of amino acids could not be found on the official website of the National Center for Biotechnology Information (NCBI). Moreover, this sequence could not be found in blood cells of 101 healthy individuals or in the 12,935 kinds of TRB sequences that could combine with viruses from the genomic database. Therefore, this TRB sequence may be

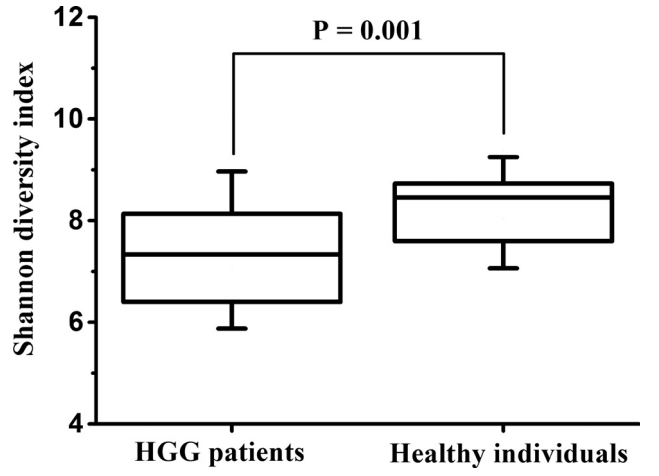


Figure 1: The Shannon diversity index of T-cell receptor of blood cells in patients with HGG is much lower than that of healthy individuals. Median: 7.34 *vs.* 8.45; Mean value: 7.07 ± 0.28 *vs.* 8.04 ± 0.14. HGG: High-grade glioma

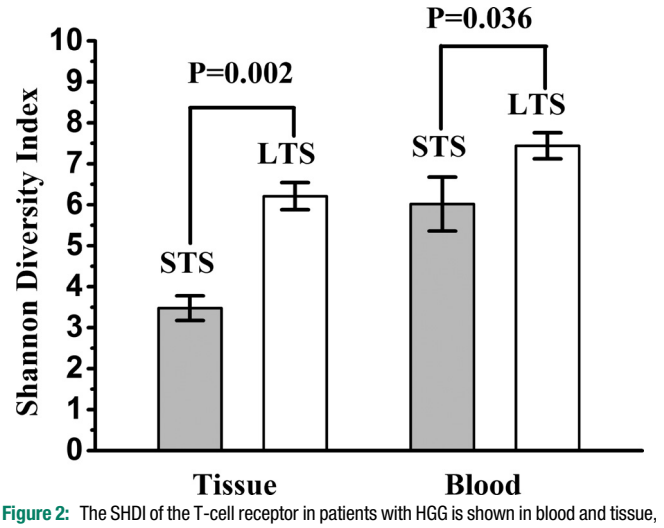


Figure 2: The SHDI of the T-cell receptor in patients with HGG is shown in blood and tissue, and SHDI of LTS patients is prominently higher than that of STS patients. LTS denotes patients who survived more than 16 months. HGG: High-grade glioma; LTS: Long-term survival; SHDI: Shannon diversity index; STS: Short-term survival.

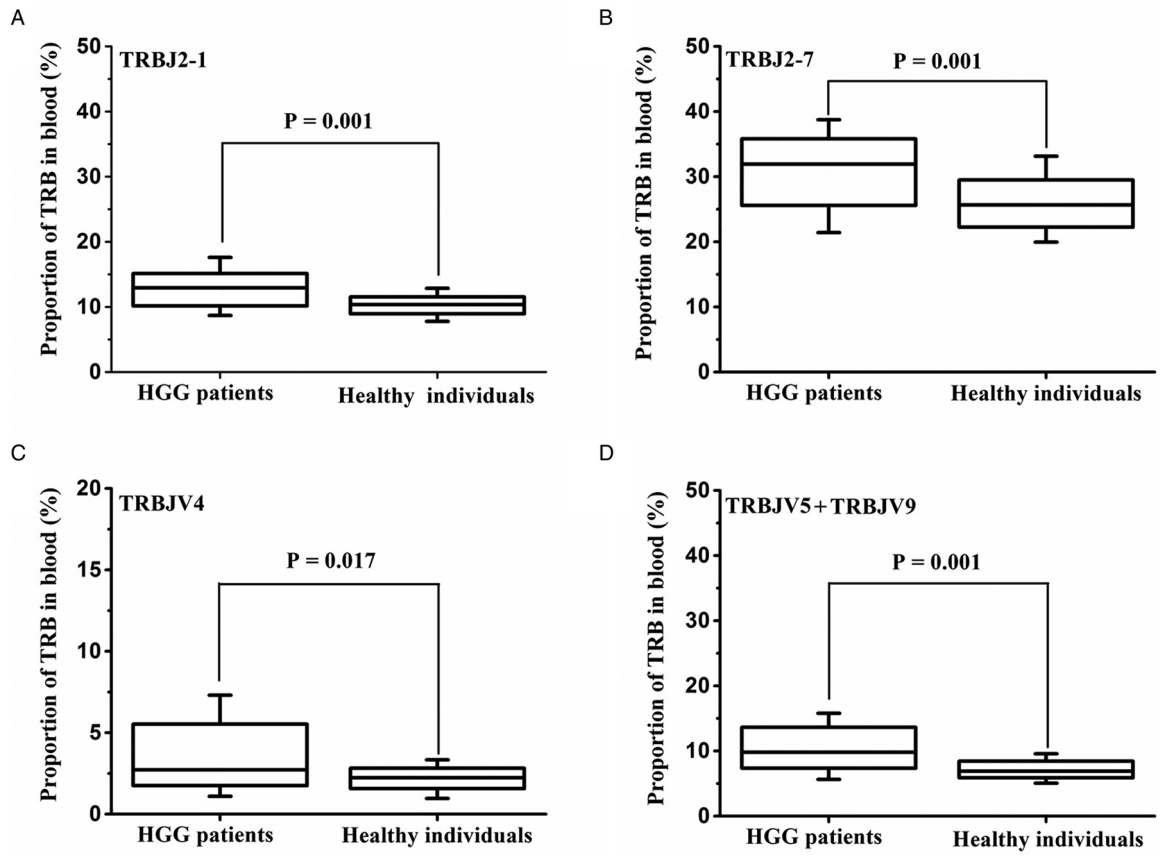


**Figure 3:** HGG patients achieve a distinctly higher proportion level than that of healthy individuals in the proportion of TRBJ2-1 (A), TRBJ2-7 (B), TRBV4 (C), and TRBV9 + TRBV5 (D) regions. HGG: High-grade glioma; TRB: T-cell receptor beta chain.

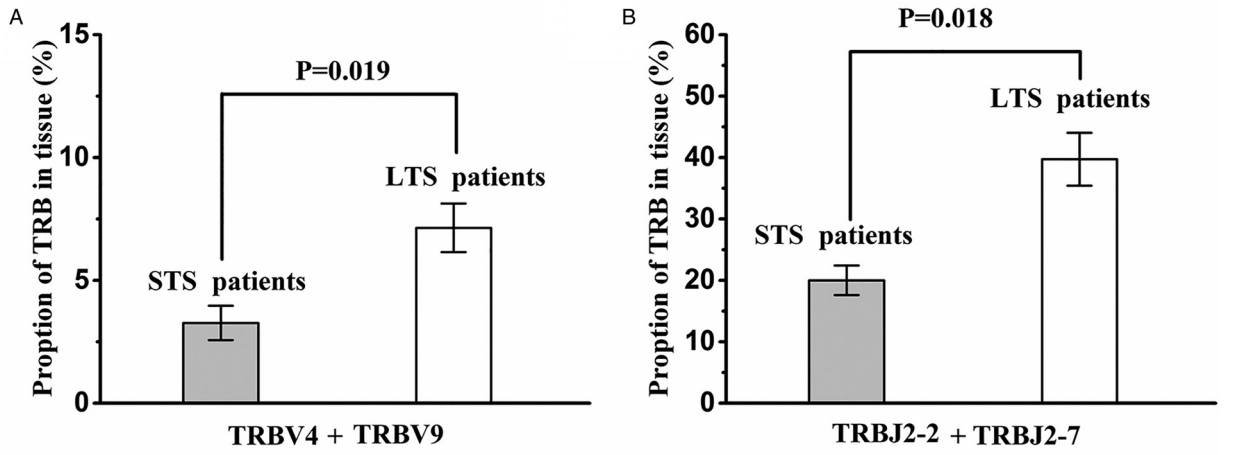


**Figure 4:** In surgical tissue, patients who survived for more than 16 months (LTS) obtained an obvious higher percentage of TRBV12 and TRBV9 + TRBV4 than patients with shorter survival times (STS) (A). Between LTS and STS patients, the sum proportion of J2-2 and J2-7 in surgical tissue also varied remarkably (B). LTS: Long-term survival; STS: Short-term survival; TRB: T-cell receptor beta chain.

one of the reasons why the two GBM patients survived more than three times longer than the median survival time of all GBM patients.

## Discussion

In recent years, advances in multiple PCR and high-throughput sequencing technology have made it possible to easily analyze the TCR repertoire.[30] These advanced technologies were applied in our current study, which yielded reliable results. The SHDI of TRB sequences in HGG patients was much lower than that of healthy individuals, indicating that a low diversity of TRB sequences may be associated with the pathogenesis of HGG patients. Meanwhile, in both tumor tissues and blood cells, the SHDI of TRB in HGG patients who survived more than 16 months was prominently higher

than that of HGG patients with shorter survival times. This result indicates that a high diversity of TCR may be beneficial for LTS of HGG patients; alternatively, the features of HGG patients' TRB sequences may be more pronounced in surgical tissue than in blood cells. This result could be ascribed to various bacterial and/or viral infections in the blood. Recently, a study showed that the lack of T cells in GBM might limit antitumor capacities and may represent a tumor-adaptive mode of T-cell dysfunction.[31] The results of this study were consistent with the results of our present study and reveal some related mechanisms. In addition, some studies have indicated that TCRs are also shared by natural-killer T cells and that there is potential cross-talk between these two populations.[32,33]

In tumor tissue, we found that patients who survived more than 16 months obtained an obvious higher percentage of TRBV9 + TRBV4, indicating that the TRBV9 region in TCRs may be a protective factor for LTS of HGG patients.

When it comes to the same TRB sequences among different HGG patients, in surgical tumor tissue, we found a special TRB sequence expressed in two GBM patients who survived more than 46 months, with both TRBV9 and TRBJ2-1 regions residing within this sequence. In addition, this sequence was not detected in 101 healthy specimens or in 12,935 different species of TRB sequences that could bind to the virus. Therefore, this TRB sequence may be one of the reasons why the two GBM patients survived much longer than other GBM patients. Moreover, the above results have provided basic information showing that this TRB sequence may be a potentially therapeutic TRB sequence.

Although T cells have been reported to play a pivotal role in a variety of tumors, there are few available therapeutic TCRs.[34-37] Due to the huge differences in TRB sequences between different individuals, studies on the same TRB sequence among patients is rare. Therefore, our research methods possessed high novelty, and our results confirmed the feasibility of screening out the same TRB from different HGG patients. Moreover, a novel approach was provided to find more potentially effective TCRs for immunotherapies, including TCR-T therapy.

In addition, because surgical specimens of GBM patients with a survival period of more than 3 years are rare, our research is more representative for LTS patients and offers future promise. Therefore, the obtained TRB sequence could be valuable for future studies and therapeutic development. Moreover, after being further confirmed and artificially modified, this TRB sequence may be applied in clinical treatments for prolonging the survival of patients or making individuals immune to cancer cells in the future. Moreover, compared with most immunotherapeutic methods that require individualized treatment, our research provides the same TRB sequence that can be used for different tumor patients; this may enable more patients to receive effective treatment in time. Finally, this study was limited by the relatively small sample sizes, so extending these experiments to larger sample sizes to further verify the current result is ongoing in our laboratory.

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## Conflicts of interest

None.

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