Characteristics of profiling the peripheral blood T-cell receptor repertoire in patients with diffuse large B-cell lymphoma

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Research Article

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphoma with strong heterogeneity and high invasiveness. The high-throughput sequencing of peripheral blood T cell receptor (TCR) can analyze the individual's immune response and directly reflect the immune status. This study aimed to detect the TCR β chain in peripheral blood of DLBCL patients and the control group, and also to analyze the characteristics of the TCR CDR3 immune repertoire of DLBCL patients before and after chemotherapy. We found that the diversity of the baseline TCR repertoire in DLBCL samples were significantly reduced than those in the control group, while there was no difference in diversity before and after chemotherapy. Patients with 53.5 years or older, stage III-IV, high IPI score and ECOG performance showed a limited TCR CDR3 repertoire. The baseline TCR diversity of patients who achieved complete remission (CR) was higher than that of the non-remission (Non-CR) group. Comparing the V gene and J gene of the CR group and the Non-CR group, it was found that the two Vβ genes have the potential to predict the therapeutic effect. In addition, standard first-line chemotherapy regimen caused changes in the frequency of T cell clones in DLBCL patients. The similarity index of CR group was lower, indicating that more changes in TCR clones occurred after chemotherapy. This study revealed the unique TCR CDR3 immune repertoire of DLBCL patients and its relationship with clinical characteristics. Dynamic monitoring of TCR diversity can more accurately predict clinical benefit.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma (NHL), accounting for 25–35% of NHL(1). It has the characteristics of strong heterogeneity and high invasiveness, and patients typically present with rapidly enlarging lymphadenopathy. By using the standard first-line treatment regimen of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP), 50–70% of DLBCL patients can be cured. The 5-year overall survival rate was 60–70%(2–4). However, about 30%-40% of DLBCL patients relapse after achieving remission or eventually develop into refractory disease. Only a minority of patients achieved long-term remissions after being given high-dose salvage chemotherapy and autologous stem cell transplant(5).

T cells are involved in the elimination of cancer cells through traditional therapies such as chemotherapy and radiotherapy, as well as immunotherapy. In-depth analysis of T cells may understand the process of tumorigenesis and development or anti-tumor immune response(6, 7). T cell-mediated antigen recognition depends on the interaction of T cell receptor (TCR) and antigen major histocompatibility complex (MHC) molecules. In most T cells, TCRs are composed of either alpha-beta (αβ) or gamma-delta (γδ) chains, and the variable region is encoded by multiple noncontiguous variable (V), diversity (D), and joining (J) gene segments(8). The rearrangement of V, D, and J genes allows the generation of various highly variable complementarity determining region 3 (CDR 3) to recognize different antigens.

The traditional methods for detecting TCR CDR3 immune repertoire are flow-cytometry and immunoscope spectratyping technique(9–11). With the development of high-throughput sequencing technology, in-

depth detection and quantification of TCR have been achieved. High-throughput sequencing technology can simultaneously analyze the CDR3 sequences of all T cell receptors, quantify the use of V and J, and fully reflect the diversity and differences of the TCR CDR3 immune repertoire.

The quantitative detection of the diversity and clonality of the TCR CDR3 region can directly reflect the function of T cells and the immune response. In this study, we used high-throughput sequencing technology to analyze the unique characteristics of TCR CDR3 in DLBCL patients and their relationship with clinical characteristics, and further explored the effect of chemotherapy on the TCR CDR3 repertoire.

Materials And Methods

Patients

A total of 30 newly diagnosed DLBCL patients from the First Affiliated Hospital of Zhengzhou University were included into this study. All patients received rituximab combined with CHOP, and peripheral blood samples were collected at baseline and four cycles after chemotherapy. This study was approved by the Institutional Review Board of the First Affiliated Hospital of Zhengzhou University (2018 – 103). We collected clinical information from hospital case systems and assessed clinical responses, including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Clinical characterization of these patients was summarized in Table 1. Peripheral blood samples from 30 healthy, age-matched volunteers who had no recent infection, fever, or history of autoimmune disease or malignancy were used as controls.

Table 1
Characteristics of 30 DLBCL patients

N	**30**
Median age	53.5(21–77)
Gender	
Male	18
Female	12
Dignosis	
GCB	11
Non-GCB	19
Ann Arbor stage	
I-II	16
III-IV	14
IPI score	
0–1	17
>1	13
Bonemarrow involvement	
Yes	3
No	27
Ki67	
<=70%	18
>70%	12
HBV	
Yes	8
No	22
DLBCL, diffuse large B-cell lymphoma; IPI, International Prognostic Index; GCB, germinal center B-like; non-GCB, Non-germinal center B-cell like.	

High-throughput sequencing

We isolated PBMC (peripheral blood mononuclear cells) from 10ml peripheral blood samples using Ficoll density gradient method, and extracted RNA for reverse transcription using QIAGEN single-step RT-PCR kit (catalog number 210212). The study used specific primers to obtain TCR variable region polynucleotides for sequencing to obtain sequence information. After high-throughput sequencing, the data were analyzed using the ImMunoGeneTics information system (http://www.imgt.org/IMGT_vquest/share/textes/)(12) to allocate the V, D and J genes. Highly variable CDR3 sequences were identified and translated to identify conserved amino acids in the CDR3 region.

TCR repertoire diversity and similarity between samples

The diversity of the TCR repertoire can be described and quantified by two independent factors: Clonotype (the number of unique CDR3 sequences) and the Shannon index. Shannon entropy is calculated based on the clonal abundance of all unique CDR3 sequences. However, considering the comparison between samples with different total number of sequencing reads, the normalized Shannon entropy (Shannon index) established by dividing the Shannon entropy by the natural logarithm of the number of unique productive TCR sequences(13). The greater Shannon index value indicates the higher TCR diversity of samples. We used the D50 value to simplify the description of diversity. The different CDR3 types obtained by sequencing are recorded as X types, which constitute the total number of N CDR3 sequences. Sort by copy number from most to least, the number of clone types occupying 50% of the total sequence is recorded as H. The ratio of H to X is D50. The D50 value is positively correlated with diversity (Table S1).

The Bhattacharyya coefficient is used to determine the similarity between two samples. It is based on the frequency and homogeneity of shared TCR sequencing reads in the two samples. The range is 0–1, where 0 means there is no overlap between the two TCR repertoires, and 1 means that the TCR repertoires between the two samples is the same(14).

Statistical Analysis

The Mann-Whitney U test was used to compare the differences between the two groups, and the Mann-Whitney. Spearman rank test was used to analyze the correlation between variables. A receiver operating characteristic (ROC) curve is a graphical plot that illustrates the diagnostic ability of a binary classifier(15). Using SPSS 23.0 to calculate all statistical analyses, *P* value of < 0.05 was considered statistically significant.

Results

Analysis of TCR CDR3 sequencing data

Sequencing data were analyzed from blood samples of DLBCL patients and healthy controls. A total of 873937 unique TCR CDR3 amino acid sequences were obtained, with an average of 14565.6 per sample. The number of unique CDR3 in the patient group was lower than that in the control group ($P < 0.0001$),

and the median value was 11412 (9071.25 ~ 16014.75) and 16691 (15159.5 ~ 18890), respectively. The V gene of the patient group (50, range from 50 to 52) was lower than that of the control group (52, range from 51 to 54), which was statistically significant (P = 0.0006) (Table 2). In addition, the median number of 502 V-J pairs (range from 479.5 to 516.75) identified from the DLBCL patient group was lower than that of healthy controls (537, range from 518.75 to 560.75) (P < 0.0001), indicating antigenic stimulation and certain specific V-J combination amplification.

Table 2
Sequencing data of TCR CDR3 repertoires for DLBCL patients and controls

	Patient	**Normal**	***P***
Unique CDR3	11412(9071.25 ~ 16014.75)	16691(15159.5 ~ 18890)	< 0.0001
Unique V family	50(50 ~ 52)	52(51 ~ 54)	0.0006
Unique J family	13(13 ~ 14)	13(13 ~ 14)	0.188
V-J pair	502(479.5 ~ 516.75)	537(518.75 ~ 560.75)	< 0.0001

All 57 V genes and 14 J genes were observed, and the frequency of these gene usages was analyzed in each sample. The 27 V genes and 3 J genes between DLBCL patients and controls were significantly differences by Mann-Whitney U test. TRBV28, TRBV18, TRBV12-3, TRBV27, TRBV14, TRBV7-7, and TRBV12-4 were more common in patients, while others were used more frequently in the control group (Table S2). Among all J genes, only TRBJ2-3, TRBJ1-4, and TRBJ1-2 showed different frequency of use between the two groups, and the control group was higher (Table S3).

Comparison of the diversity of TCR repertoires between DLBCL patients and controls

We used Shannon index, unique clonotypes and D50 to quantify the diversity of the TCR repertoires. Compared with the control group, the D50 and the number of clonotypes in DLBCL patients (Fig. 1B, 1C) were significantly lower (P < 0.0001; P < 0.0001). However, no difference was observed in the three indicators before and after treatment.

The relationship between TCR CDR3 diversity and clinical characteristics

We assessed the relationship between TCR CDR3 diversity and clinical characteristics in DLBCL patients. Patients with stage III-IV had less TCR CDR3 diversity than patients with stage I-II (*P* = 0.003). Compared with younger patients, patients with a median age of 53.5 years or older had lower D50 values (*P* = 0.023). Regarding the IPI score and ECOG performance, the D50 value of patients with a score greater than 1 decreased (*P* = 0.036) (Fig. 2). In order to study how the diversity of peripheral blood TCR reflects the immune status of DLBCL, we evaluated the relationship between lactate dehydrogenase (LDH) and β-2 microglobulin and CDR3 diversity, and found their negative correlation (Figure S1).

The relationship between TCR diversity and clinical response

According to the efficacy evaluation after four cycles of treatment, DLBCL patients were divided into CR group and Non-CR group. The TCR diversity of non-CR group before treatment was lower than that of CR group (Figure S2). Since TCR V and J genes show different usages between individuals, we continue to investigate whether the use of TRBV and TRBJ genes has potential therapeutic efficacy predictors in patients. We compared the use of V and J genes in the blood before treatment between the two groups, and found that in the CR group with good clinical response, TRBV5-1, TRBV6-1, TRBV6-7, TRBV6-9, TRBV7-4, TRBV7- 7. The frequency of TRBV11-1 and TRBJ2-5 is significantly higher. Then we established a Logistic regression model to predict the efficacy. The model was evaluated by the receiver operating characteristic curve (ROC). As a result, the combined prediction model including TRBV7-7 and TRBV11-1 classified the efficacy prediction (AUC = 0.891) (Fig. 3).

The high TCR similarity index is related to the poor efficacy of DLBCL patients

In order to quantitatively evaluate whether the change in the distribution of clones is treatment-induced TCR repertoires reconstruction or treatment-induced new clones generation, we applied the Bhattacharyya coefficient to calculate the similarity index between the pre- and post-treatment samples. Our study showed that after effective treatment in DLBCL patients, there was a significant correlation between the similarity index and the response to treatment, and the similarity index of the CR group decreased(Figure 4A, 4B, P = 0.048, AUC = 0.712).

To further describe the frequency of all TCR clones pre- and post- treatment, we use pre-treatment/post-treatment clones as the X/Y axis. TCR clones with the same frequency are on the Y = X diagonal, and the identified new clones or changed clones are located above or below the diagonal. The frequency of T cell clones increased or decreased significantly in each treated patient, and more significant changes were observed in CR patients (Fig. 6D). Next, we evaluated the increase and decrease of TCR clones in patients with DLBCL pre- and post- treatment. In 30 patients, the median percentages of expanded and contracted TCR clones were 12.18% and 14.73% (Fig. 6C), indicating that the percentages of increased and decreased TCR clones after treatment were similar.

Discussion

T cells can specifically recognize a variety of antigen molecules including neoplastic antigens through different types of TCRs, which can activate the immune response(16). The highly variable CDR3 is the key region for TCR to recognize foreign antigens(17). Therefore, analyzing the diversity of the CDR3 receptor library can directly reflect the function of T cells and the state of immune response, which is essential for evaluating the response of patients to treatment.

High-throughput sequencing technology can perform large-scale detection and quantitative analysis of TCR libraries(18). Wu et al. used high-throughput sequencing technology to monitor MRD in patients with acute T lymphoblastic leukemia (T-ALL)(19). Oki et al. used high-throughput sequencing to detect classic Hodgkin's lymphoma (cHL) patients and observed that specific gene sequences in tumor tissues can also be detected in the corresponding peripheral blood samples(20). Tumeh et al. found that patients with

advanced melanoma who were effective in PD-1 inhibitor treatment had a significant increase in TCR diversity and TCR clonality in tumor tissues after treatment(21).

In this study, through high-throughput sequencing of the TCR repertoire of DLBCL patients and healthy controls, it was found that the two groups showed different characteristics in unique CDR3 types, VJ gene frequency, and V-J pairing. According to the TCR diversity score-D50 value, the diversity of the patient group was significantly less than that of the control group. Among different clinical characteristics, we found that patients with age greater than the median age of 53.5 years, stage III-IV or IPI score greater than 1 have lower TCR repertoires diversity, which is related to poor immune status. The levels of LDH and B-2 microglobulin in patients with DLBCL were negatively correlated with TCR diversity. LDH is a key enzyme for tumor metabolism, and elevated LDH is a marker of immunosuppression in cancer (22). The concentration of blood B-2 microglobulin reflects the activation level of the cellular immune system, and has been proven to be an important prognostic factor for multiple histological types of lymphoma(23–25). These data indicate that the TCR repertoires in peripheral blood samples of DLBCL patients can be clearly distinguished from healthy individuals in several aspects, and the characteristics may reflect tumor-related immune status. The immune status of patients with low TCR diversity may be severely impaired.

In addition, we analyzed the different uses of V and J genes between the CR group and the Non-CR group. There are 8 genes with significant differences between the two groups. Among them, TRBV7-7, TRBV11-1 and their binary logistic regression predictive values can reflect the clinical efficacy of patients. Correspondingly, previous studies have shown that the TRBV gene is related to the clinical response of membranous nephropathy(26) and can also predict the prognosis of non-small cell lung cancer(27). The diversity of TCR in peripheral blood of tumor patients is associated with clinical efficacy. Severe restriction of TCR diversity in peripheral blood of breast cancer patients at baseline is associated with poor prognosis (28). In this study, the diversity of TCR pools in the CR group was higher than that in the Non-CR group. High TCR diversity may indicate a better clinical response. The high diversity of peripheral blood TCR pools allows individuals to have more possible tumor-specific T cells to limit immune escape. These specific T cells can control the growth of cancer cells and recognize the corresponding antigens after entering the tumor site(29). Therefore, increasing the diversity of TCR is necessary to improve anti-cancer immunity.

Chemotherapy can induce the remodeling of TCR pool in peripheral blood of patients with DLBCL. We compared the similarity between samples pre- and post- treatment and found that the similarity between samples of patients who achieved CR was lower than that of patients with PR. At the same time, the frequency of all TCR clonotypes of patients pre- and post- chemotherapy was described. Compared with the other group, the CR group produced more new clones and changed clonotypes, indicating that patients with better clinical response may have a lower similarity index. This trend may be due to clonal proliferation of T cell clone subtypes that recognize tumor neoantigens during treatment. Furthermore, we found that the percentage reduction of TCR clonotypes pre- and post- treatment was similar to the percentage increase, indicating that chemotherapy not only induced clonal contraction of high-frequency

clones in peripheral T cells, but also induced clonal expansion of low-frequency clones. Yin et al. used T cell high-throughput sequencing on 15 CLL patients treated with ibrutinib and found that the diversity of TCR in the peripheral blood of the patients increased significantly after treatment. In addition, a variety of low-frequency clonotypes and active specific clonotypes increased significantly(30).

In summary, this study analyzed the basic characteristics and clinical significance of the TCR repertoire in diffuse large B-cell lymphoma, and identified two baseline V gene frequencies that are related to treatment efficacy. Patients with good clinical response have higher TCR diversity. In addition, dynamic monitoring of TCR, such as the similarity index pre- and post- chemotherapy may more accurately predict clinical benefit. However, this result is probably not enough to explain the immune response of DLBCL patients, and a limited number of patients may make these results unstable. It is necessary to conduct further prospective studies on larger samples. Finally, considering the T cell dysfunction displayed by DLBCL patients, when clinicians design new treatment strategies, they also need to consider restoring the patient's anti-tumor T cell immunity.

Declarations

Acknowledgments

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Data Availability statement

The datasets generated or analyzed during this study are available from the corresponding author on reasonable request.

Ethics approval

This study was approved by the Institutional Review Board of the First Affiliated Hospital of Zhengzhou University (2018-103). Written informed consent was obtained from all the participants prior to the enrollment of this study

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Authors’ contributions

Jing Li wrote the main manuscript text, Zhiyuan Zhou, Zhixin Zhang and Xiaorui Fu prepared figures and tables. Mingzhi Zhang reviewed the manuscript.

Conflict of Interest

All authors declare that there is no conflict of interest in this study.

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Figures

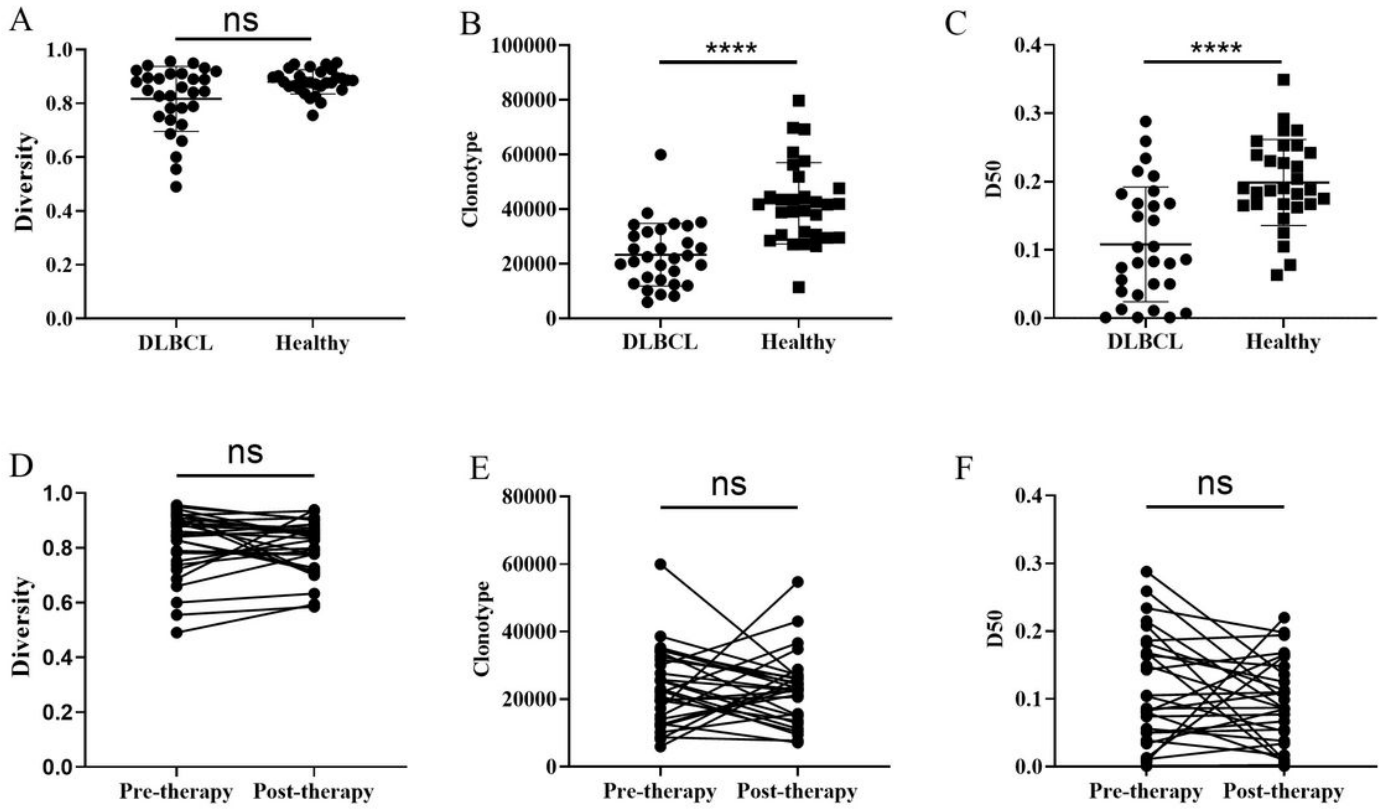

Figure 1

Comparison of diversity, number of clonotypes and D50 between DLBCL patients and healthy controls (A, B, C). Statistical analysis was performed using the Mann-Whitney test. Comparison of diversity, number of clonotypes and D50 of patients before and after treatment (D, E, F). The data was analyzed using the Wilcoxon matched-pairs signed rank test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$,****$p < 0.0001$. ns, no significant.

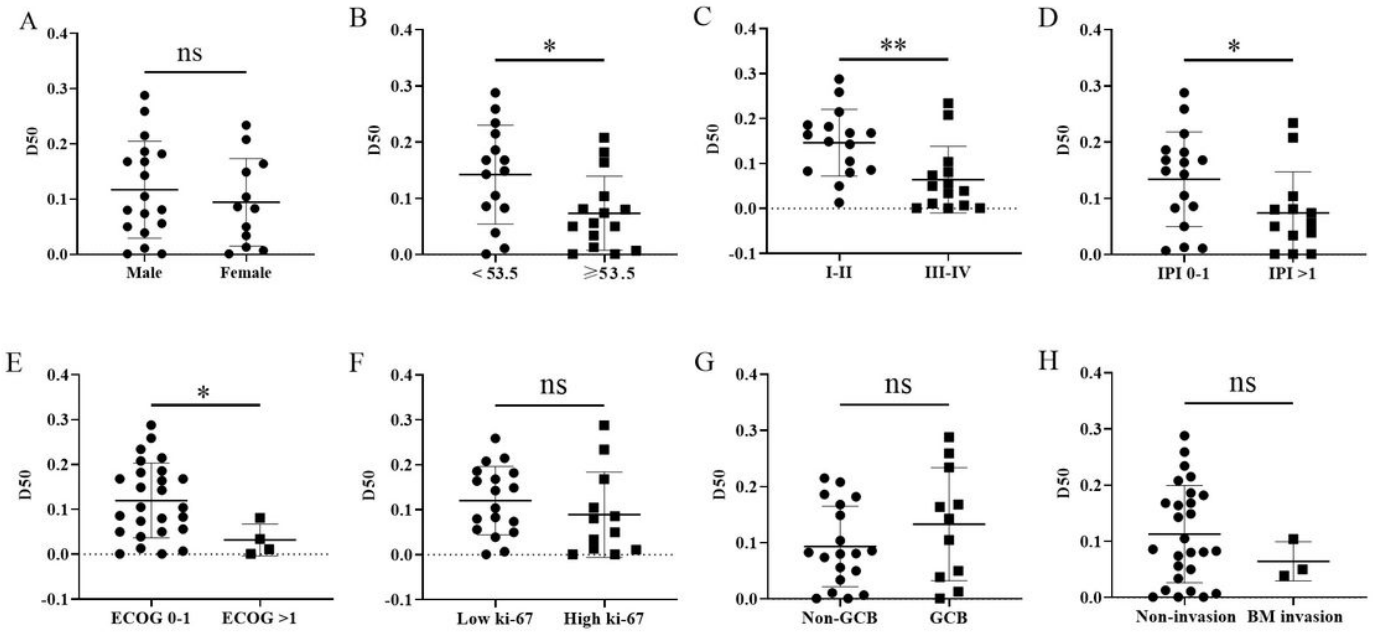

Figure 2

The relationship between the clinical characteristics of DLBCL patients and the D50 of TCR CDR3. *p < 0.05, **p < 0.01, ***p < 0.001,****p < 0.0001. ns, no significant.

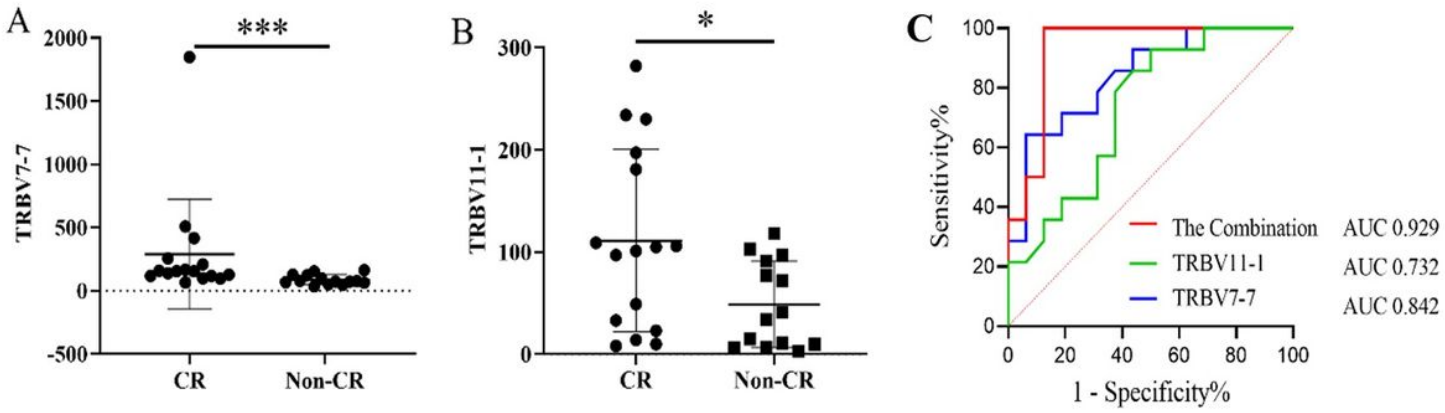

Figure 3

The usage frequencies of TRBV7-7 and TRBV11-1 gene segments in CR group and non-CR group (A, B). ROC analysis for the combined prediction model of TRBV7-7 and TRBV11-1 gene segments in DLBCL patients to separate the two groups(C). *p < 0.05, **p < 0.01, ***p < 0.001,****p < 0.0001. ns, no significant.

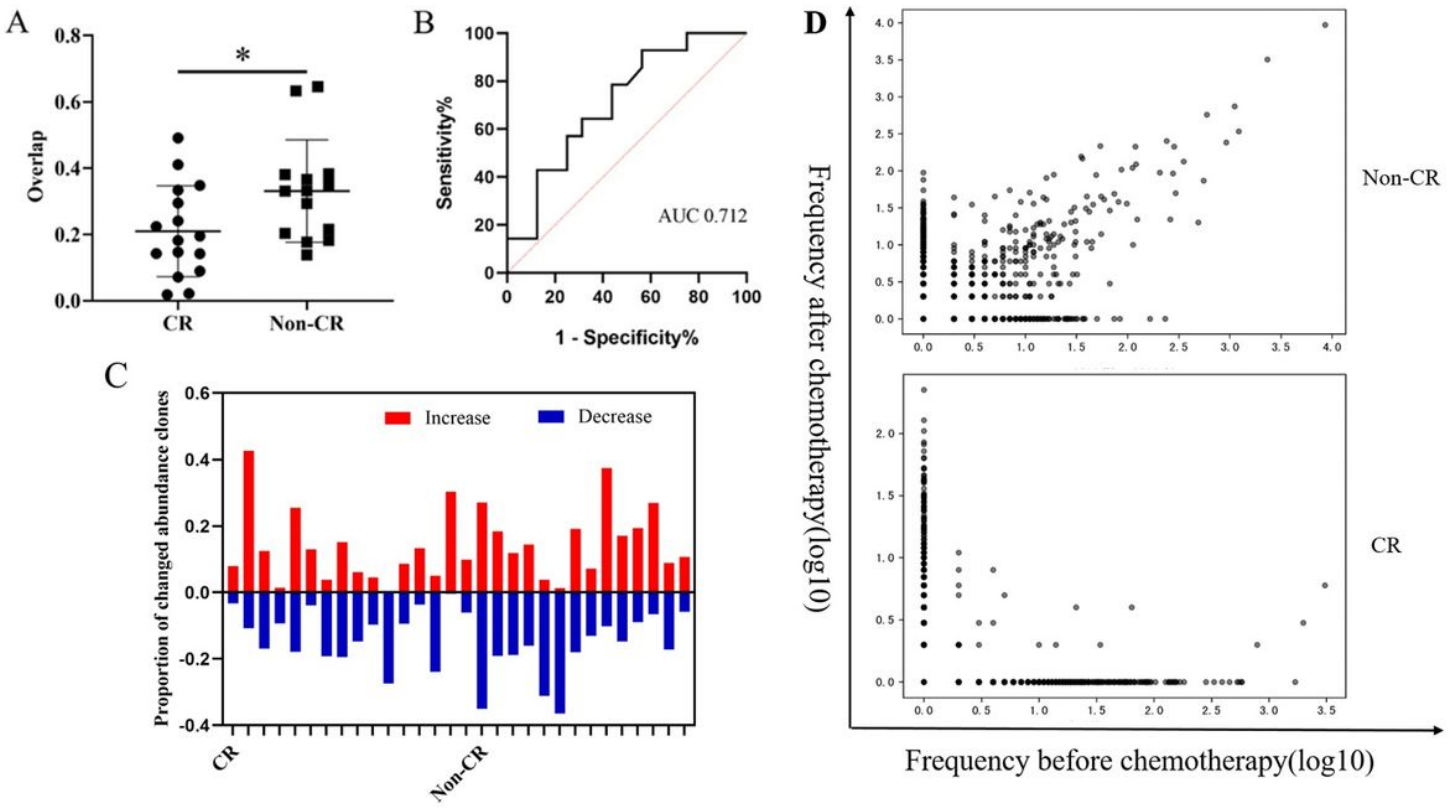

Figure 4

TCR CDR3 repertoires reconstruction of DLBCL patients before and after chemotherapy. Comparison of the similarity index (Overlap) between the CR group and the Non-CR group (A). Analyze the ROC curve of the similarity index between the samples to separate the CR group and Non-CR group (B). The proportion of changed abundance clones in the two groups (C). The frequency of all TCR clones before and after treatment (D).

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