Characteristics of profiling the peripheral blood T-cell receptor β-chain repertoire in gastric cancer

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Keywords: T-cell receptor (TCR) repertoire, CDR3 diversity, gastric cancer

Posted Date: April 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-20212/v1

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Abstract

Background: Gastric cancer is the third cause of cancer-related deaths worldwide, and is initially detected and attacked by the immune system through tumor-reactive T cells. The aim of this study was to determine the basic characteristics of the peripheral blood T-cell receptor (TCR) repertoire in patients with gastric cancer.

Methods: High throughput sequencing was used to identify hyper-variable rearrangements of complementarity determining region 3 (CDR3) of the TCR β chain to comprehensively profile the TCR repertoire in peripheral blood samples from 6 advanced gastric cancer patients and 3 early gastric cancer controls.

Results: The study showed that the TCR repertoire differed substantially between advanced cancer patients and early controls in terms of CDR3 clonotype, diversity and V/J segment usage. Specifically, low diversity reflected a worse immune status and prognosis in advanced gastric cancer. However the diversity of TCR was not significant difference in wild or mutation patients.

Conclusion: TCR repertoire analysis served as a useful indicator of disease development and prognosis in gastric cancer and may be utilized to be biomarker for immunotherapy.

Background

Gastric cancer is the third cause of cancer-related deaths worldwide, and the poor prognosis of patients is largely due to the high frequency of tumor recurrence or metastasis within 24 months after surgical resection[1, 2]. Many studies have demonstrated that multiple factors participate in the occurrence and development of gastric cancer. The majority of human solid tumors are infiltrated by lymphocytes which has been as a biomarker to predict the immune responses, tumor progression or the efficacy of therapy[3–6]. T cells, as vital factors in the anticancer immune response, participate in the cancer-cell killing activities of immunotherapy as well as chemotherapy and radiotherapy. Immature T cells recognize antigen peptide via specific T-cell receptors(TCRs), a heterodimer composed of α and β variant chains, expressed on the cell surface,thereby activate into mature T cells. The specificity of T-cell responses to antigens are largely depended on the affinity of TCR:peptide-MHC (pMHC) complexes[7, 8]. In response to the enormous amount of foreign antigens, it is crucial to develop and maintain a highly diversified TCR repertoire. The specificity and diversity of TCRs are predominantly originated from the highly variable complementarity determining region 3 (CDR3)[9, 10]. CDR3, the only non-germline coding region of the T cell receptor, is generated by random rearrangement and junction region mutation of V(D) J regions located in TCR-coding genes[11, 12]. CDR3 is the hot spot for peptide contact and the determinant for the specificity and affinity of antigen recognition. The frequency of the CDR3 region gene sequence can reflect the functional status of the TCR[12]. And several evidences have shown that CDR3 diversity and clonal enrichment are important in cancer diagnosis, therapy, and Prognosis[13–16]. Thus,
the number of TCR clones can indirectly reflect the features of T cells. Therefore, in-depth analysis of TCR has the potential to provide essential insight for understanding an individual’s tumor and immunity[17].

However the knowledge of the TCR patterns in gastric cancer is still limited. The TCR repertoire is heterogeneous, differing between regions within the same tumor in gastric cancer and many other malignancies. Moreover, the existence of differences in TCR patterns between tumor tissues or blood samples highlight the necessity of additional detailed studies for specific samples. TCR diversity in blood samples can be used to monitor disease progression and immunotherapy response[18], a better understanding of the impact of gastric cancer on TCR diversity is important. Therefore, in our study, we implemented the first systematic analysis of CDR3 diversity of TCR β chains in blood from advanced stages gastric cancer patients and early stages controls to depict the diversity of peripheral blood T-cell repertoire and to investigate the TCR diversity associated with clinical characteristics in gastric cancer.

Materials And Methods

Subject Cohorts

Peripheral blood samples were obtained from 9 patients with gastric cancer (3 early stages and 6 advanced) who were hospitalized for anticancer treatment in the department of oncology, zhongda Hospital(jiangbei), Medical School, southeast University (Nanjing, China). Peripheral blood samples from 3 early stages were as controls. Subjects with past or concurrent autoimmune disease or AIDS were excluded from the study. Peripheral blood was collected at the beginning of the first cycle of treatment. Circulating tumor DNA (ctDNA) was used to detect tumor mutational burden. Clinical information was collected from the hospital’s case management system. Durable clinical benefit (DCB) was defined as disease-free survival (DFS) beyond 24 months. The study was approved by the Institutional Review Board of Zhongda Medical College of southeast University. All subjects provided written informed consent.

RNA Isolation and High-throughput sequencing of T-cell receptor β genes

Total RNA was isolated from peripheral blood using the TRlzol reagent according to the manufacturer’s protocol. The concentrations of RNA were evaluated using a NanoDrop ND-2000 spectrophotometer (Thermo Scientific). A total of 200 ng RNA was converted into cDNA by reverse transcription using a Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science, Penzberg, Germany) according to the manufacturer’s protocol on a T100TM Thermal Cycler (Bio-Rad Inc., CA, USA). Then the libraries were constructed following the protocol from Liang et al[19].

For TCRβ CDR3 library preparation, two-round nested amplicon arm-PCR was performed with a Multiplex PCR Assay Kit Ver. 2 (TaKaRa, Dalian, China) using specific primers against each variable and constant gene. PCR products were purified by agarose gel electrophoresis, amplified using Illumina sequencing
primers with different sample barcodes, and subjected to high-throughput sequencing using the Illumina HiSeq X Ten platform with a read length of 2 × 150 bp.

Data Processing and Analysis

Raw FASTQ files were subjected to Illumina adaptor trimming, then the paired-end reads containing ambiguous bases (N) or poor quality (15% nucleotide positions with a Phred quality < 30) were removed by a custom script in Perl. The high-quality reads were used for subsequent analysis. The V-, D- and J-genes were identified using BLAST+ (version 2.7.1) by aligning to their reference sequences in the international ImMunoGeneTics (IMGT) information system (IMGT: http://www.imgt.org/). CDR3 TCR sequences were identified as previously reported[20, 21]. The CDR3 was extracted for each read as the nucleotide sequence between the conserved TCR V cysteine and TCR J phenylalanine residues. Since CDR3 sequences including frameshifts or stop codons cannot translate into a functional protein, only productive reads that do not contain frameshifts or stop codons were used for analyses. The diversity of TCRβ was measured by Gini coefficient, normalized Shannon diversity entropy and Rank-abundance, which have been widely used for assessing the richness and diversity of TCR as previously described . Circular plots were created using Circos (version 0.69-6) with the darkness of the links and broadness of the Bézier curve range reflecting the number of jumping pairs. (The primers, virtual probes sequences and the PCR conditions are not publicly available because are currently protected by intellectual property rights of the authors, in prevision of their potential future commercial exploitation.

ctDNA Extraction

The blood was centrifuged in Streck Cell-free tubes at 1600 g for 10 minutes at 4 °C and supernatant was transferred to a 1.5 ml Eppendorf tube, followed by 4 °C at 16000 g for 20 minutes to remove residual cells and debris. The supernatant of second centrifugation was collected for ctDNA extraction using QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions. DNA concentrations were determined by the Qubit dsDNA HS Assay Kit (Thermo Fisher). ctDNA libraries were constructed with the KAPA Hyper Prep Kit (Roche) according to the manufacturer's protocol. The concentrations and size distributions of the libraries were respectively analyzed by Qubit and Caliper. A customized set of biotinylated DNAs probe was used to capture targeted DNAs for plasma ctDNA with SeqCap EZ Accessory Kit V2 and SeqCap Pure Capture Bead Kit (Roche) following the standard protocols. Capture probe was designed to cover coding sequencing or hot exons frequently mutated in solid tumors. The captured DNAs were amplified by PCR, and the final DNA concentrations and sizes were respectively measured by Qubit and Caliper. The captured libraries for plasma ctDNA were loaded into the NextSeq 500 (Illumina) for 75 bp paired-end sequencing according to the manufacturer's instructions.

Statistical analysis

Differences between groups were compared using the Mann–Whitney or Kruskal–Wallis tests. Correlations between variables were analyzed using Spearman's rank test. Relationships between clinical benefit and the TCR repertoire were determined using Fisher’s exact test. All statistical analyses were
calculated using GraphPad Prism 7.0, or SPSS 24.0. A two-sided p value < 0.05 was considered statistically significant. All data were analyzed using two-tailed tests.

Results

Subject characteristics

A total of 9 samples from 9 gastric cancer patients, who were 6 advanced stage and 3 early stage controls were analyzed in this study. The pathological classifications of all tumor is adenocarcinoma. The 6 advanced gastric cancer contained lymph node metastasis and vascular invasion and so on. Anticancer treatment included chemotherapy and surgery therapy. These therapies were often used in combination in most patients. Early stage patients (33.3%) had received Oxaliplatin combined with S-1, and advanced stage patients (66.7%) had received Docetaxel combined with S-1. All the patients received the high throughput sequencing and two of nine patients were detected P53/PTEN and PI3K mutation respectively. All the patients received the biochemical detects as the baseline examination. Detailed patient characteristics and outcomes are summarized in Supporting Information Tables 1 and 2.

Differences in TCR repertoires between early and advanced stage patients

Diversity of the TCR repertoire can be measured using the V-J and the CDR3 clonotype. We then compared TCR diversity between two groups. We identified a total of 50 distinct V gene segments and 13 distinct J gene segments from every sample. Of these fragments, the most frequent V gene segments were TRBV7-2 (23.11% early stage, 14.91% advanced stage) and TRBV7-8 (18.76% early stage, 14.24% advanced stage) were more expression in patients with early gastric cancer, whereas other fragments were more expression in advanced stage gastric cancer controls. Five of the most frequent J gene segments were TRBJ1-5 (24.75% ) — TRBJ 2–2 (23.68%,p = 0.05) — TRBJ1-6 (13.95%) — TRBJ2-5 (12.89%) and TRBJ2-6 (1.88%) in advanced gastric cancer, whereas other fragments were more expression in early stage gastric cancer (Fig. 1A). As shown in Fig. 1B, a heat map was generated according to the usage frequency of V and J gene segments. Furthermore, we also detected the composition of V-J gene combinations and found a total of 671 distinct V-J gene combinations. V-J paired fragments exhibit more diversity in early stage gastric cancer group than advanced stage group (Fig. 1C, p = 0.028). Further, The composition of V-J gene combinations revealed significant usage differences in two groups, including TRBV3-2_TRBJ1-1 — TRBV7-3_TRBJ1-1 — TRBV7-8_TRBJ2-3 — TRBV5-8_TRBJ1-5 (Fig. 1D, P ≤ 0.05). Compared with early and advanced stage, the expression of V-J gene combinations were different in two groups (sFig. 1A and 1B). CDR3 clonotypes, which include a conserved cysteine in the V region and a conserved phenylalanine in the J region, determine the diversity of the TCRβ repertoire. We first investigated CDR3 variety and expression in two groups and found that advanced gastric cancer patients showed significantly lower V-D-J and CDR3 variety compared to early controls (Figs. 1E and 1F). The different expression levels of V-D-J and CDR3 were analyzed by crater plots. As shown in Figs. 1G and sFig 1C,
there were differences in the expression levels of some segment combination between advanced and early gastric cancer. Analysis of V-D-J fragments revealed that 6 VDJ fragments exhibited differential expression between two groups (sFig. 1D). Furthermore, Rank-abundance and normalized Shannon diversity entropy were used to compare CDR3 diversity. All results suggested a remarkably decreased CDR3 diversity in advanced groups (Fig. 1H and sFig. 1E,F). These data demonstrated that the TCR repertoire in peripheral blood samples from gastric cancer patients could distinguish between early and advanced gastric cancer in several aspects. The advanced stage changed the usage of several key low-frequency CDR3 and lessened TCRβ diversity. The patients with advanced gastric cancer exhibit limited TCR repertoire diversity and that this diversity is further limited in patients with advanced stage. Moreover, these changes may implied an altered immune status associated with gastric cancer stage.

The prognosis value of TCR repertoires in the advanced gastric cancers

Next we continued to explore the prognostic value of the TCR repertoire. To determine whether patients with poor prognosis demonstrated differences in diversity and gene expression level, we identified six advanced stage patients. Among B1 and B3 who have longer DFS (37.2 and 26.5+ months respectively) were as control group. To be similar, we identified a total of 48 distinct V gene segments and 13 distinct J gene segments from every sample. Of these J fragments exhibited different usage frequency between two groups, TRBJ2-2 was 49.04% in control group whereas 11.00% in case group. TRBV7-2 and TRBV7-8 counted of 49.92% in control group whereas 19.96% in case group (Fig. 2A). As shown in Fig. 2B, a heat map was generated according to the usage frequency of V and J gene segments. Further, The composition of V-J gene combinations revealed significant usage differences in two groups, TRBV5-6_TRBJ1-2 and TRBV6-1_TRBJ1-5 showed more frequency in control group especially TRBV6-1_TRBJ1-5 combinations; however, TRBV19_TRBJ 1–4 showed lower frequency in control group (Fig. 2C and 2D). However, cluster analysis of the V-J and V-D-J fragments showed that 4 VDJs and 2 VJ fragments showed different expression levels between shorter DFS gastric cancer patients and longer controls (Fig. 2E and sFig.2A). The difference in expression levels of V-J / V-D-J and CDR3 fragments was then analyzed by crater plots and heatmap. As shown in Figs. 2F and 2G, there was a difference in the amount of gene expression in gastric cancer with a long DFS, especially that some fragments of the CDR3 region have abnormally high expression(sFig. 2C). In addition, V-J paired also showed different expression between groups (sFig. 2B). These data suggest that peripheral blood CDR3 and VDJ expression provide valuable prognostic information. Differential expression of TCR profiles in peripheral blood samples of patients with advanced gastric cancer may predict prognosis. This results indicated that not only the ratios of common T-cell diversity gradually decreased, but their frequencies also gradually changed, some high-frequency TCR clones turned into low-frequency ones, while some lesion specific clones abnormally amplified.

Mutation and TCR repertoires among the gastric cancer
All the patients received the ctDNA detect and NGS detect. Among these patients there were 2 patients were detected P53, PI3K and PTEN mutation (table 2), therefore the two patients were as the control group compared to other patients. We identified a total of 48 distinct V gene segments and 13 distinct J gene segments from every sample. Of these fragments, The most frequent J gene segments were TRBJ 1–6 (41.82% mutation group, 0.78% wild group) and TRBJ 2–7 (22.85% mutation group, 4.74% wild group) were more frequencies in patients with mutation group, whereas other fragments were more expression in wild group; however, the frequent of V gene segments was similar in mutation and wild groups (Fig. 3A). Figure 3B demonstrated a heat map which was generated according to the usage frequency of V and J gene segments. As shown in sFigure 3A and 3B, there were differences in the expression levels and preferential usage of some J genes fragments between mutation and wild patients. The analysis of V-J and V-D-J fragments revealed that VDJ and VJ fragments combination exhibited differential usage frequencies between mutation and wild gastric cancer patients (Fig. 3C and sFig3C). Then the analysis of the differences in V-J/V-D-J and CDR3 expression was performed by the volcano plot. As shown in Fig. 3D-E and sFig3D there were difference expression in mutation and wild gastric cancer, especially CDR3 have down expression in wild gastric cancer; however there was no significant diversity of V-D-J and CDR3 clonotype in two groups (data not shown). These data demonstrate that the somatic mutation in peripheral blood samples from patients is not relation with clonotype number and diversity; however, the there is relevant with expression and usage frequencies.

Discussion

The immune system is becoming the most powerful and complex anticancer weapons since 2015 [22, 23]. The immune checkpoint inhibitors (PD-1/PD-L1 antibody or CTLA-4 antibody) and engineered immune cells (chimeric antigen receptor (CAR)-T cells or neoantigen-specific T cell receptor (TCR)-T cells) in the application of cancer therapy indicated the beginning of a new area in medicine. The FDA has approved Pembrolizumab was applied for advanced gastric cancer with second-line treatment failure[24]. However, only a subset of people exhibit efficient immune responses to immunotherapy[25], suggesting that the identification of elements of cancer immunity and immune profiles merits contribute to predicting the therapeutic response for tumor eradication.

Tumor infiltrating T lymphocytes have been shown to play a critical role in the anti-tumor immune response. The TILs of tumor is likely to be a prognosis for different solid tumors regardless of the stage [26]. However peripheral T cells shuttle back and forth between the tumor and systemic circulation[27]. Approximately 95% of circulating mature T cells in the peripheral blood utilize the T cell receptor (TCR) for specific recognition of tumor antigens. High diversity of the peripheral TCR repertoire limits the extent of immune escape by increasing the more tumor-specific T cells, which recognize the corresponding antigens to control the tumor cells growth[28, 29]. Therefore the diversity of the TCR repertoire can reflect the clonal expansion of T cells and the tumor burden and the status of the patient’s immune function [30, 31]. Differences in infiltrating TCR repertoires between normal and cancer tissues have been reported in
several studies\[14, 32–34\]. In fact, immune responses to tumor antigens have been shown to be significant effect on disease progression in gastrointestinal cancers\[35\]. TCR of an individual can be accurately profiled using deep sequencing\[36\]. In this study, we analyzed the TCR repertoire of peripheral blood cells from patients with advanced gastric cancer and early gastric cancer controls. These two groups showed differing TCR repertoires in terms of CDR3 variety, V-D-J segment variety and expression. Compared to early gastric cancer individuals, patients with advanced gastric cancer had lower CDR3 and V-D-J paired diversity and expression. Further analysis revealed that V-J and V-D-J paired usage frequency was difference in patients with a more severe disease state. The functions of these genes need to be further investigated. Taken together, our study evaluated TCR varieties in gastric cancer (including early and advanced) for the first time to explicate the multistage heterogeneity of the TCR varieties during gastric malignant transformation. These results indicate that decreased diversity in the peripheral TCR repertoire could reflect the gastric cancer stage and progression. Our results provided a new strategy to discover potential prognostic biomarkers by integrating analysis of TCR repertoire changes and the special molecular in the progression of cancer.

Observation of the frequency and expression of TCR in advanced gastric cancer provided evidences for the progression and prognosis related immune response in the immune system. This discovery may provide new insights into the immune regulatory function of the immune system. In summary, our work was the first to demonstrate the frequency and expression of the TCR\(\beta\) immune response during advanced gastric cancer progression and impaired immune status, which at least partially explained the rapid progression of patients with locally advanced gastric cancer. It provides novel insights into the relationship between the prognosis of gastric cancer and the immune system.

Cancer is a genetic disease caused by the accumulation of somatic cell mutations. Neoantigens as a direct consequence of somatic mutations within tumour cells could promote to activate tumour antigen-specific T cells. The interplays between mutated tumor cells and the immune system play a vital role throughout stages of tumorigenesis. In the lately studies circulating tumor DNA(ctDNA) and TCR sequencing are emerging approaches for identifying corresponding mutations from the primary tumor and characterizing of the T-cell repertoire\[37\]. The somatic alterations detected in ctDNA are directly derived from an individual tumor. Accordingly, ctDNA mutations can be used as biomarkers which identify potentially “driver genes” as well as detect residual disease or monitor tumor levels during therapy\[38\]. CtDNA combined with TCR can effectively monitor the body's specific immune response during anti-PD-1 drug treatment. An study showed that the lower diversity in patients with a mutated TP53 gene indirectly reflects a possible positive correlation between CDR3 diversity and PD-L1 expression\[39\]. This correlation also supports the biomarker function of CDR3 diversity. However, in the present study, we did not observed the correlation between ctDNA and diversity of TCR in peripheral blood. The diversity of TCR did not showed distinction between mutation and wild gastric cancer; however, the usage frequencies and expression of TCR were significant difference between mutation and wild patients. The mechanism of TCR diversity is caused by somatic cell rearrangement, nucleotide diversity and epigenetic alterations. The V-D/D-D/D-J/V-J junction regions of TCR genes are separated by several nucleotides which are determined by the terminal nucleotide transferase\[11\]. The other possible
reason was that we used the small panels of 65 genes to detect ctDNA not the whole genome sequencing for detection ctDNA.

**Conclusion**

Our study assessed the peripheral blood TCR repertoires of gastric cancer (including early and advanced) to elucidate the multistage heterogeneity of TCR repertoire during gastric malignant transformation. Moreover, we determined the association between the variation in the TCR repertoires and the stage of gastric cancer. Although the effects of these genes on the behaviour of gastric cancer cells remain unclear and the results presented require further exploration, our study reported a novel way of discovering biomarkers for gastric cancer prognosis and improving the comprehension of immune responses during carcinogenesis. Without a doubt we need the more efforts to explore the mechanisms that lead to the changes in the TCR repertoire during gastric tumourigenesis.

**Abbreviations**

T-cell receptors  TCRs  
Peptide-MHC  pMHC  
Complementarity determining region 3  CDR3  
Circulating tumor DNA  ctDNA  
Durable clinical benefit  DCB  
Disease-free survival  DFS  
Chimeric antigen receptor  CAR  
Programmed death 1  PD-1  
Programmed cell death Ligand 1  PD-L1

**Declarations**

Ethics approval and consent to participate

The research protocol was reviewed and approved by the Human Research Ethics Committee of zhongda Hospital, and written informed consent was obtained from each patient included in the study.

Consent for publication

All authors give consent for the publication of the manuscript.
Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information file. Further details are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No.81773284).

Authors' contributions

YWLand YHS study concept and design. NNG: acquisition of data. YTW: analysis and interpretation of data. YWL: writing the manuscript. The author(s) read and approved the final manuscript.

Acknowledgments

Not applicable

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**Supplementary Figure Legends**

**sFigure1.** A-D Expression of V-J V-D-J and CDR3 by the heatmap and Volcano. E Comparison of CDR3 aa diversity by Rank-abundance. F Hierarchical clustering was performed based on similarities of CDR3 aa sequences and visualized using heatmaps.

**sFigure2.** A-B Expression of V-J V-D-J and CDR3 by the heatmap and Volcano. C Heatmap of CDR3 aa clonetype between prognosis well and poor. Heatmap demonstrated the frequencies of the most abundant CDR3 clonetype.
Figure 3. A-B: Expression of J by the heatmap and Volcano. C-D: Expression of V-J by the heatmap and Volcano.

Tables

Table 1

| Group | Patient | Gender | Age | Size of tumor [cm³] | Lymph node metastasis | Vasculature invasion | Differentiation | Stage |
|-------|---------|--------|-----|---------------------|-----------------------|----------------------|-----------------|-------|
| A     | A1      | male   | 65  | 2*2*1.3             | 0                     | Positive             | Moderately      | □A    |
|       | A2      | male   | 64  | 2*2*2.3             | 0                     | Negative             | Poorly          | □B    |
|       | A3      | male   | 66  | 3.3*2.7*            | 1                     | Negative             | Moderately      | □B    |
| B     | B1      | female | 69  | 10*7*2              | 5                     | Positive             | Poorly          | □A    |
|       | B2      | male   | 64  | 3*3*1               | 30                    | Positive             | Poorly          | □B    |
|       | B3      | male   | 49  | 7*7*2.5             | 2                     | Positive             | Poorly          | □C    |
|       | B4      | male   | 64  | 4.5*4*1.3           | 4                     | Positive             | Well            | □B    |
|       | B5      | male   | 66  | 5*4*1               | 4                     | Positive             | Poorly          | □C    |
|       | B6      | male   | 69  | 3.8*2.5*0.7         | 8                     | Positive             | Moderately      | □B    |

Table 2

| Patient | Chemotherapy | DFS [m] | ctDNA | ctDNA ng/μl | ALC | N/L R | LDH | Albumin | CD4 | CD8 | CD4/CD8 |
|---------|--------------|---------|-------|-------------|-----|-------|------|---------|-----|-----|---------|
| A1      | OXA/S-1      | 24+     | Wild  | 1.57        | 1.68| 1.38  | 143  | 43      | 556 | 184 | 3.02    |
| A2      | OXA/S-1      | 24+     | Wild  | 1.32        | 1.26| 2.07  | 162  | 40.8    | 724 | 336 | 2.15    |
| A3      | OXA/S-1      | 24+     | Wild  | 1.14        | 1.66| 1.46  | 171  | 35.1    | 376 | 496 |         |
| B1      | TAX/S-1/+S-1 | Maintain | 37.2  | Wild        | 0.778| 1.19  | 1.37 | 204     | 39.8| 376 | 204     | 1.84    |
| B2      | TAX/S-1/+S-1 | Maintain | 12    | Wild        | 0.31 | 1.27  | 1.72 | 145     | 41.6| 224 | 216     | 1.04    |
| B3      | TAX/S-1/+S-1 | Maintain | 26.5  | Wild        | 0.896| 2.69  | 0.59 | 166     | 43.6| 408 | 276     | 1.48    |
| B4      | TAX/S-1/+S-1 | Maintain | 16.7  | PI3K        | 0.43 | 1.34  | 0.90 | 166     | 38.6| 252 | 252     | 1.00    |
| B5      | TAX/S-1      |         |       | P53         | PTEN| 0.648 | 0.38 | 9.24    | 220 | 41.3| 296     | 152     | 1.95    |
| B6      | TAX/S-1/+S-1 | Maintain | 15.0+ | Wild        | 0.735| 1.62  | 1.47 | 900     | 720 | 120 | 1.25    |

B4_PIK3CA exon21  c.3139C>T p.H1047Y  0.24%
B5_APC  exon9  c.901C>A p.P301T  3.53%
TP53  exon6  c.578A>G p.H193R  10.38%

Figures
Figure 1

Differences in TCR repertoires between early and advanced stage patients. (A) The mean distribution of all identified V and J gene segment usage. (B) The heatmap of J (upper) and V (down) gene usage between early and advanced groups. (C) V-J paired variety in advanced gastric cancer and early controls. (D) The frequency of V-J segment usage that had significant differences (P<0.05). (E) Varieties of V-D-J segments and TCR CDR3s were compared between advanced patients and early controls. (F) Expression of V-D-J segments was compared between advanced patients and early controls. (G) Comparison of CDR3 aa diversity by the simpson index.
Figure 4

The prognosis value of TCR repertoires in the advanced gastric cancers. (A) The mean distribution of all identified V and J gene segment usage in 6 advanced patients. (B) The heatmap of J (left) and V (right) gene usage between prognosis poor and well groups. (C) The frequency of V-J segment usage that had significant differences (P<0.05). (D-E-G) Expression of V-D-J segments and CDR3 were compared between prognosis poor and well controls.
Figure 7

Mutation and TCR repertoires among the gastric cancer cases. (A) The mean distribution of all identified V and J gene segment usage between mutation and wild groups. (B) The heatmap of V (left) and J (right) gene usage between two groups. (C-E) Expression of V-D-J segments and CDR3 were compared between mutation and wild controls.

Supplementary Files

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