ONCOLOGY LETTERS 28: 413, 2024

Exploring the potential of the TCR repertoire as a tumor biomarker (Review)

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Received February 29, 2024; Accepted June 14, 2024

DOI: 10.3892/ol.2024.14546

Abstract. T cells play an important role in adaptive immunity. Mature T cells specifically recognize antigens on major histocompatibility complex molecules through T-cell receptors (TCRs). As the TCR repertoire is highly diverse, its analysis is vital in the assessment of T cells. Advances in sequencing technology have provided convenient methods for further investigation of the TCR repertoire. In the present review, the TCR structure and the mechanisms by which TCRs function in tumor recognition are described. In addition, the potential value of the TCR repertoire in tumor diagnosis is reviewed. Furthermore, the role of the TCR repertoire in tumor immunotherapy is introduced, and the relationships between the TCR repertoire and the effects of different tumor immunotherapies are discussed. Based on the reviewed literature, it may be concluded that the TCR repertoire has the potential to serve as a biomarker for tumor prognosis. However, a wider range of cancer types and more diverse subjects require evaluation in future research to establish the TCR repertoire as a biomarker of tumor immunity.

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Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; ICIs, immune checkpoint inhibitors; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; PD-Ls, programmed cell death ligands; V, variable gene fragment; D, diversity gene fragment; J, joining gene fragment; CDRs, complementary determining regions; NGS, next-generation sequencing; PCR, polymerase chain reaction; 5'-RACE, 5'-rapid amplification of cDNA end; HCC, hepatocellular carcinoma; CC, cervical cancer; PDAC, pancreatic ductal adenocarcinoma; NSCLC, non-small cell lung cancer; PSCC, penile squamous cell carcinoma; RCC, renal cell carcinoma; TILs, tumor-infiltrating lymphocytes; TME, tumor microenvironment; CAR, chimeric antigen receptor; MIBC, muscle-invasive bladder cancer; HGSOC, high-grade serous ovarian cancer

Key words: TCR repertoire, biomarker, tumor diagnosis, tumor immunotherapy, tumor prognosis

Contents

1. Introduction

Cancer develops due to a series of genetic changes in normal cells, which causes them to become malignant (1) with the ability to invade surrounding normal tissues and metastasize. Immunotherapy is a promising treatment method for tumors. The most studied immunotherapy involves immune checkpoint inhibitors (ICIs). ICIs function by targeting specific proteins expressed on immune or cancer cells, thereby alleviating inhibitory signals that prevent the immune system from attacking cancer cells and augmenting the immune response against these cells (2). Key checkpoint proteins, including cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and programmed cell death ligands (PD-Ls), serve as primary targets for ICIs. By inhibiting these checkpoint proteins, ICIs enhance the immune-mediated attack on malignant cells (3). Additionally, various other immunotherapies, including sipuleucel-T, have been clinically employed for cancer treatment. Sipuleucel-T is an immunotherapy that was approved in 2010 for the treatment of advanced prostate cancer. It is a personalized treatment designed to stimulate the immune system of the patient, causing it to target and attack prostate cancer cells (4).

It is important to determine the effect of treatment and the prognosis after treatment, and biomarkers can help to achieve these goals. Due to some patients with cancer being unable to undergo surgery or biopsy, the identification of non-invasive cancer biomarkers is urgently necessary.

A number of studies have focused on the characteristics of T cell receptor (TCR) repertoires, and have demonstrated that they differ among cancer tissues, adjacent tissues and peripheral blood (5) and may be different after treatment than they were before (6). As the characteristics of certain TCR repertoires are associated with prognosis (7), TCR repertoires have potential as biomarkers. Therefore, it is important to understand the characteristics of the TCR repertoire in different locations in the body and at different time points in patients with cancer.

2. TCR structure

The immune system comprises adaptive and innate components. The innate immune system protects the body from general pathogenic factors, whereas the adaptive immune system targets and is able to remember specific pathogens (8).

T cells are key components of the adaptive immune system that recognize pathogens or abnormal peptides and specifically initiate adaptive immunity. T cells function by either directly killing infected cells (9) or releasing cytokines to attract other immune cells. This activity is triggered when T cells recognize foreign antigens displayed on the surface of antigen-presenting cells through the major histocompatibility complex (MHC) (10). This display allows TCRs on T cells to identify specific peptides or antigen epitopes, which initiates the T-cell response (Fig. 1A). TCRs are highly diverse heterodimers composed of α and β chains, or γ and δ chains, encoded by TRA, TRB, TRG and TRD genes, respectively (Fig. 1B). The ab heterodimers constitute the majority of these TCRs, accounting for ~95% of the total number of TCRs, and can identify MHC-presented antigen peptides or antigen epitopes. By contrast, the γδ TCRs are less abundant, with only 1-5% of T cells expressing them; moreover, they are involved in the innate immune response and are not restricted to antigens presented by MHC molecules. However, the ligands to which γδ T cells bind are unclear (11). TCR genes contain variable regions (V regions) and constant regions. The variable region is assembled by variable (V), diversity (D) and joining (J) gene fragments through an orderly process known as V(D)J recombination, in which one allele of each gene fragment is randomly recombined with other gene fragment alleles to form a functional antigen recognition region (12,13) (Fig. 1C). The V region of each TCR chain consists of three highly variable complementary determining regions (CDRs), namely CDR1, CDR2 and CDR3, CDR3 has the highest variability of these CDRs and is the most important region for specific antigen recognition (14) (Fig. 1B).

The CDR3 region is responsible for binding antigenic peptides presented by MHC molecules (15,16). Due to its direct interaction with antigenic peptides and high variability, which enables the recognition of diverse antigens, the CDR3 region provides a wealth of knowledge about TCR specificity.

3. TCR repertoire analysis

The development of next-generation sequencing (NGS) has revolutionized the characterization of immune libraries, allowing for large-scale parallel TCR sequencing. A wide range of computing and mathematical tools has been created to model and describe the diversity of these libraries. NGS is advantageous in that it has greater sequencing depth and quantifies TCR clonal abundance with markedly higher accuracy than is possible by spectratyping, in which the number of clonotypes is determined based on the number of different CDR3 lengths (17). Compared with single-cell TCR sequencing, population TCR sequencing is more commonly used in the study of TCR diversity, and facilitates the analysis and comparison of different repertoires in tumors.

The TCR repertoire may be analyzed using both genomic DNA (gDNA) and RNA (18). Although DNA is highly stable, its analysis has low sensitivity and the presence of alleles may affect sequencing accuracy. By contrast, although RNA is less stable than DNA, RNA analysis is more sensitive and can eliminate allele interference (15). Following the selection of the analyte for TCR analysis, a library must then be prepared. Commonly used library preparation methods include multiplex polymerase chain reaction (PCR) and 5' rapid amplification of cDNA end (5'-RACE) (19).

gDNA and RNA are both suitable starting materials for multiplex PCR, and can be used for multiple rounds of PCR. These multiple rounds of PCR may introduce sequencing bias and error, potentially leading to some alleles being more easily amplified, thus affecting the accuracy of the results (20,21). However, this can be corrected, e.g., by changing primer concentrations (22). RNA can also be analyzed using 5'-RACE (23), which uses a reverse transcriptase with terminal transferase activity to reverse transcribe RNA while untemplated sequences, mainly including deoxycytidine triphosphate are added at the 3' end. A template switch oligonucleotide containing a complementary poly(G) strand binds to the 3'-terminal sequence of the first strand and initiates the chain reaction (24). Almost all the cDNA fragments that are obtained via this method remained intact. Therefore, only a pair of primers are required for subsequent template amplification to achieve complete amplification of the possible V gene.

In the analysis of the TCR diversity index, metrics such as Shannon entropy and clonality are commonly used to quantify TCR diversity and the expansion of specific TCR clones within a sample. Shannon entropy effectively quantifies the complexity and breadth of the TCR repertoire by evaluating how uniformly the different TCR variants are distributed within the T-cell population. The formula introduces the TCR diversity index, H(X), as a measure of TCR diversity, as follows:

$$\mathrm{H(X)} = -\sum_{i=1}^{n} \mathcal{P}(x_i)\log_e(\mathcal{P}(x_i))$$

In the formula, $P(x_i)$ denotes the relative frequency or probability of occurrence for TCR variant i in the sample. In this context, $P(x_i)$ indicates the proportion of a specific TCR variant relative to the entirety of distinct TCR variants, where n represents the aggregate count of unique TCR variants that are identified. Consequently, Shannon entropy serves as an indicator of TCR

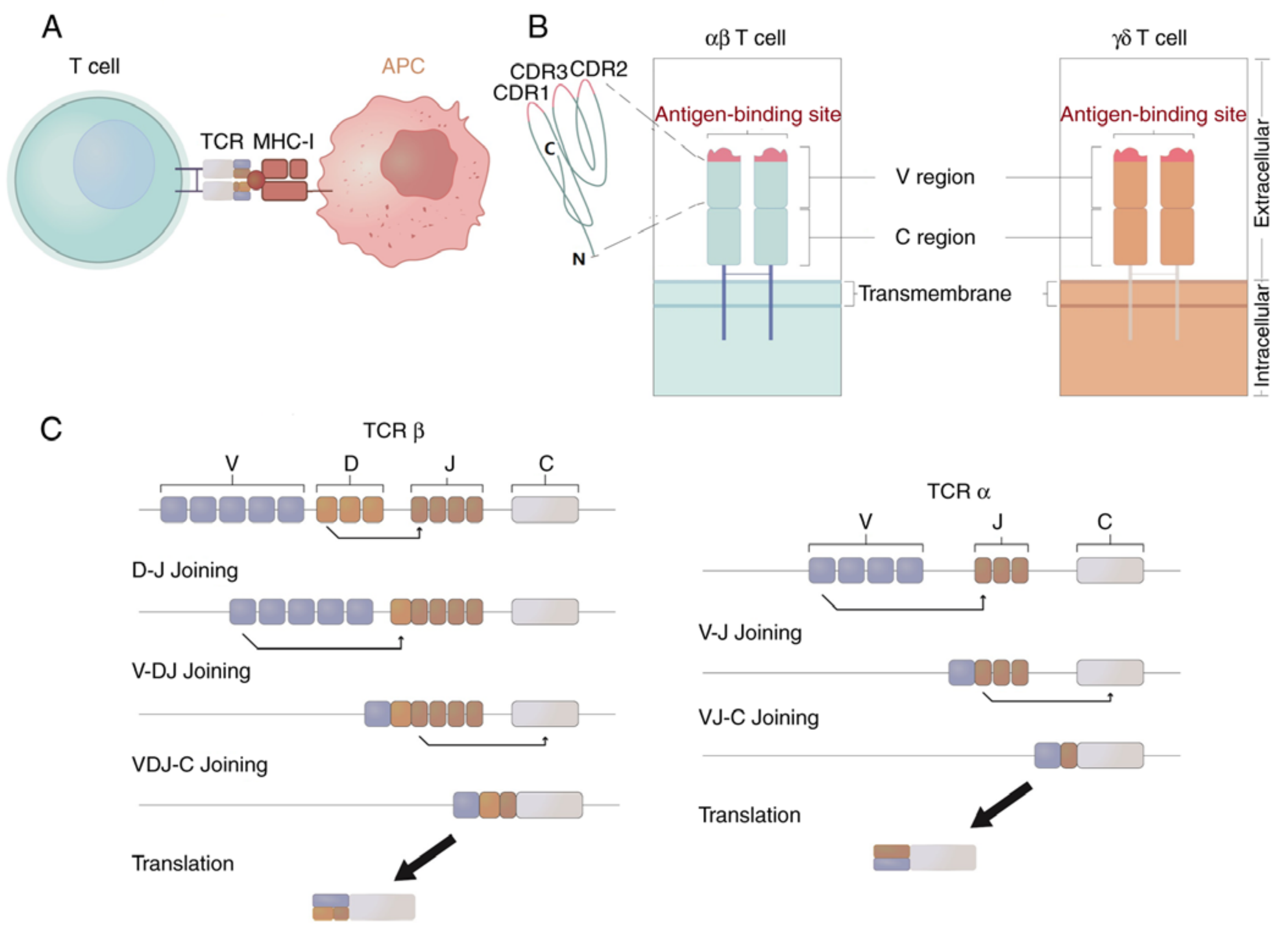

Figure 1. TCR classification, rearrangement and MHC interaction. (A) Interaction between the TCR and MHC. (B) On the T-cell surface there are α-chain and β-chain paired TCRs and γ-chain and δ-chain paired TCRs. The V region of a TCR comprises three highly variable complementary determining regions: CDR1, CDR2 and CDR3. (C) Recombination of the α and β chains. During VDJ recombination of the β chain (left panel), a D fragment is randomly recombined with a J fragment, after which the D-J fragment is recombined with a random V fragment. The complete variable region is connected to the C region to encode a normally functioning β chain. During VJ recombination of the α chain (right panel), a V fragment is randomly recombined with a J fragment, after which the V-J fragment links to the C region to encode the α chain. Blue, orange, and brown colors represent V, D and J gene fragments, respectively, in the variable region. TCR, T-cell receptor; MHC, major histocompatibility complex; CDR, complementary determining region; V, variable; D, diversity; J, joining; C, constant; APC, antigen-presenting cell.

diversity; specifically, a higher Shannon entropy value signifies a greater level of diversity within the TCR repertoire (25,26). Clonality, as evaluated through standardized Shannon entropy, is delineated by the following formula:

$$C = 1 - \frac{-\sum_{i=1}^{n} P(x_i)\log_e(P(x_i))}{\log_e(n)}$$

In this formula, the variable C is a metric for the assessment of TCR clonality, which is confined to the range of 0 to 1. Essentially, TCR clonality is the extent of proliferation exhibited by specific TCR variants within a sample relative to others. A higher value of C signifies elevated clonality, which is concomitant with diminished diversity (27).

4. Application of TCRs in tumor diagnosis

Numerous studies have demonstrated that the TCR repertoire diversity in the peripheral blood or tumor tissues of patients with cancer has its own specific characteristics compared with that in healthy individuals, and may be useful in the diagnosis of various cancers.

Changes in TCR diversity in peripheral blood. In the study by Wang *et al* (5), the characteristics of the TCR repertoire in the peripheral blood of patients with hepatocellular carcinoma (HCC) were detected using high-throughput TCR sequencing. The TCR repertoire diversity in peripheral blood was demonstrated to be significantly greater than that in tumor and peritumoral tissue. In addition, it was found that patients with HCC have a unique peripheral blood TCR repertoire compared with that of healthy individuals.

However, contrasting with the findings in HCC, Cui *et al* (28) reported that the diversity of the circulating TCR repertoire in patients with cervical cancer (CC) was lower than that in healthy women and gradually decreased during tumor development. Similarly, the TCR repertoire diversity in the peripheral blood of patients with pancreatic ductal adenocarcinoma (PDAC) was shown to decline compared with that in healthy controls in a study performed by Pan *et al* (29) involving analysis of the inverse Simpson diversity index. The authors speculated that the reduced TCR repertoire diversity may be caused by the proliferation of CD8$^+$ T cells.

Changes in TCR diversity in tumor tissues. Through the TCRβ sequencing and analysis of cancer tissues and distant noncancerous tissues from 15 patients with lung cancer, Wang *et al* (30) demonstrated that the TCR repertoire diversity in lung cancer tissues was significantly greater than that in distant normal lung tissues. However, in patients with non-small cell lung cancer (NSCLC), Song *et al* (31) found that the diversity of the TCR repertoire revealed by high-throughput sequencing was not different from that of adjacent non-tumor tissues. The reason for these inconsistent results may be that the tumor microenvironment (TME) in lung cancer tissues differs among individuals, thus resulting in differences in the TCR repertoire diversity among cancerous tissues.

Changes in the TCR repertoire diversity in tumorous or normal tissues have also been observed in other types of carcinoma. In a recent study, high-throughput TCR sequencing demonstrated that the TCR repertoire diversity of HCC tumor tissues and peritumoral tissues was similar (5). This differed from the results of one previous study, in which the TCR repertoire diversity in HCC tumor tissues was higher than that in adjacent non-tumor tissues (32), but consistent with another, in which no significant difference in TCR repertoire diversity was identified between HCC tumor tissues and adjacent normal tissues (33). Sherwood *et al* (34) found that the diversity of the TCR repertoire in tumor tissues was much lower than that in peripheral blood in a study of patients with colorectal cancer. Similarly, the TCR repertoire diversity in bladder cancer tissues (35) and in penile squamous cell carcinoma (PSCC) (36) was found to be lower than that in adjacent normal tissues. By contrast, the diversity of the TCR repertoire in breast cancer tissues has been demonstrated to be greater than that in adjacent normal tissues (37). Moreover, the TCR repertoire diversity in oesophageal squamous cell carcinoma was revealed to be not significantly different from that in peripheral blood and adjacent normal tissues (38). Information on the changes in TCR repertoire observed in different cancers are summarized in Tables I and II.

The TCR repertoire diversity in the peripheral blood or tumor tissues of patients with tumors has been shown to be inconsistent compared with that in healthy individuals or adjacent normal tissues. Whether these characteristics of TCR repertoire diversity in tumors have the potential to be used as markers for tumor diagnosis requires further validation.

5. Application of TCRs in tumor therapy

Tumor immunotherapy has profoundly advanced cancer research and effectively improved the prognosis of patients with cancer (39,40), and the success of ICIs underscores the importance of the anticancer immune response in patients. The expression of biomarkers, such as PD-Ls, in cancer tissues has been shown to have an marked effect on the clinical response to ICIs. However, as tumor tissue cannot be biopsied during treatment, new biomarkers of the response to ICIs are necessary to determine the efficacy of clinical treatment. Blood samples are becoming more widely used because they are relatively easy and non-invasive to obtain compared with biopsies of tumor tissue. In addition, as numerous studies have shown that the TCR repertoire in the peripheral blood can change significantly during tumor treatment, such changes may be used as a biomarker of ICI response.

In an early study, it was reported that the TCR repertoire diversity increased after anti-CTLA-4 treatment and improved the survival of patients with metastatic castration-resistant prostate cancer or metastatic melanoma (41). Consistent with this, an increase in TCR repertoire diversity was also observed after anti-CTLA-4 treatment in another study of patients with metastatic melanoma (6). This may be attributed to the anti-CTLA-4 treatment promoting reconstruction of the TCR lineage and increasing its diversity (42). By contrast, the diversity of the TCR repertoire in peripheral blood has not been found to change significantly after anti-PD-1 therapy (43-45); however, increased TCR clonality has been observed in the peripheral blood of patients with melanoma treated with anti-PD-1 therapy (44,45). In addition, Kato *et al* (46) reported that after anti-PD-1 treatment, the clonality of the TCR repertoire in the peripheral blood of patients with advanced renal cell carcinoma (RCC) increased; however, the diversity of the TCR repertoire decreased.

Changes in the TCR repertoire have been observed after other treatments, including radiotherapy and chemotherapy. Liu *et al* (47) reported that the diversity of the TCR repertoire in the peripheral blood of patients with lung cancer was significantly lower than that in healthy individuals, with the TCR repertoire diversity decreasing after chemotherapy, radiotherapy, tyrosine kinase inhibitor therapy and/or antiangiogenic therapy. Similarly, the TCR repertoire diversity in the peripheral blood was found to decrease after FOLFIRI chemotherapy with bevacizumab or cetuximab in most patients with metastatic colorectal cancer (48), and in patients with prostate cancer after sipuleucel-T therapy (49).

The TCR repertoire of tumor-infiltrating lymphocytes (TILs) also undergoes changes. TILs are immune cells located in the TME that are crucial in the immune response against tumors (50,51). In tumors, high infiltration levels of TILs, particularly cytotoxic CD8$^+$ T cells which have the ability to kill tumor cells, are often associated with an improved prognosis (52). TILs can be used in the assessment of tumor response to immunotherapy. Tumors rich in TILs, known as 'hot' tumors, are more likely to respond to immunotherapy than are 'cold' tumors in which TILs are scarce (53). In a study of patients with rectal cancer who responded well to radiotherapy and chemotherapy, the TCR repertoire diversity of TILs was observed to increase after treatment (54). However, in a study of patients with head and neck squamous cell carcinoma, the TCR repertoire diversity of TILs was shown to be reduced, while the TCR clonotypes in TILs were expanded after cetuximab treatment (Table III) (55). Therefore, changes in the TCR repertoire of TILs with regard to diversity, clonality or clonotypes may serve as predictive therapeutic markers.

A high TCR repertoire diversity in peripheral blood is associated with an improved immune response in numerous types of tumors (56). Hopkins *et al* (57) reported that the diversity of the TCR repertoire in the peripheral blood of patients with PDAC increased after treatment. Moreover, in patients receiving anti-CTLA-4 treatment, patients whose TCR repertoire diversity increased after treatment exhibited an improved therapeutic response; however, this was not observed in patients receiving

Table I. Changes in the TCR repertoire of patients with various types of cancer, in peripheral blood.

First author, year	TCR repertoire changes			Cancer	Mean Shannon entropy, patients vs. healthy individuals	(Refs.)
Wang *et al*, 2022	Specificity -	Clonality -	Diversity ↑	HCC	13.390 vs. 10.644	(5)
Cui *et al*, 2018	Specificity ↑	Clonotypes ↓	Diversity ↓	Cervical cancer	6.830 vs. 9.943	(28)
Pan *et al*, 2023	Specificity ↑	Clonality ↓	Diversity ↓	Pancreatic ductal adenocarcinoma	5.965 vs. 6.465	(29)

HCC, hepatocellular carcinoma; ↑, increase; ↓, decrease; -, no change; TCR, T-cell receptor.

Table II. Changes in the TCR repertoire of patients with various types of cancer, in tumor tissues.

First author, year	TCR repertoire changes			Cancer	Mean Shannon entropy, tumor tissues vs. adjacent normal tissues	(Refs.)
Wang *et al*, 2019	Specificity ↑	Clonality ↓	Diversity ↑	Lung cancer	431.37 vs. 166.20[a]	(30)
Chen *et al*, 2016	Specificity ↑	Clonality ↑	Diversity ↑	Hepatitis B virus-associated HCC	0.65 vs. 0.48[b]	(32)
Wang *et al*, 2017	Specificity ↑	Clonality ↓	Diversity ↑	Breast cancer	10.928 vs. 8.870	(37)
Wang *et al*, 2022	Specificity -	Clonality -	Diversity -	HCC	10.378 vs. 10.234	(5)
Song *et al*, 2020	Specificity ↓	Clonotype ↑	Diversity -	Non-small cell lung cancer	6.850 vs. 6.737	(31)
Sherwood *et al*, 2013	Specificity -	Oligoclonality ↑	Diversity ↓	Colorectal cancer	0.84 vs. 0.88[b]	(34)
Ma *et al*, 2019	Specificity ↑	Clonality ↑	Diversity ↓	Bladder cancer	0.40 vs. 0.59[b]	(35)
Zhang *et al*, 2024	Specificity ↑	Clonality ↑	Diversity ↓	Penile squamous cell carcinoma	95.92 vs. 104.48[a]	(36)

[a]Inverse Simpson's diversity index; [b]normalized Shannon entropy. HCC, hepatocellular carcinoma; ↑, increase; ↓, decrease; -, no change; TCR, T-cell receptor.

anti-PD-1 treatment. In patients with advanced melanoma, a highly diverse TCR repertoire in the peripheral blood before treatment was found to be associated with an improved therapeutic effect of anti-CTLA-4 treatment (58,59). Notably, similar findings have also been reported for patients with other types of cancer treated with anti-PD-1 therapy. For example, in a study of gastrointestinal tumors, anti-PD-1 treatment exhibited improved therapeutic effects in patients with a higher baseline TCR repertoire diversity in the peripheral blood (43). In addition, patients with NSCLC with high TCR repertoire diversity in the peripheral blood exhibited a superior therapeutic response to anti-PD-1 treatment (60,61). Also, patients with lung cancer who had a greater TCR repertoire diversity in the peripheral blood before treatment exhibited a more favorable response to radiotherapy and chemotherapy (Table IV) (47).

The aforementioned studies demonstrate that a wider range of TCR repertoires before ICI immunotherapy is largely associated with improved clinical outcomes in patients with cancer. This may indicate that increasing the diversity of the TCR repertoire is beneficial to patients receiving ICI immunotherapy. This may be due to the increase in the diversity or clonality of the TCR repertoire, or due to the expansion of the TCR repertoire induced after ICI immunotherapy improving existing immunity, thus guaranteeing the effectiveness of immunotherapy (62). However, TCR repertoire characteristics alone are not sufficient to determine the response to tumor immunotherapy. First, tumor cells are genetically heterogeneous and undergo changes as the cancer progresses (63), which results in inability of the TCR repertoire to recognize all tumor cell variants. In addition, high-affinity TCRs may also recognize antigens expressed in healthy tissues, trigger non-targeted effects and cause autoimmune diseases (64). Second, in addition to the TCR repertoire, PD-1, PD-Ls, tumor mutational burden (65,66) and microsatellite instability (66,67) have also been shown to serve as biomarkers for tumor immunotherapy, including ICIs (68,69), chimeric antigen receptor (CAR) T-cell therapies (70), or CAR T cells combined with chemotherapy, radiotherapy or angiogenesis inhibitors (71). A comprehensive treatment strategy may combine the complementary effects of different immunotherapy methods to obtain an improved therapeutic effect. Therefore, consideration of the characteristics of the TCR repertoire in combination with those ICIs or other tumor immunotherapies may promote the further development of tumor immunotherapy.

Table III. Changes in the TCR repertoire after treatment in different cancers.

A, Peripheral blood

First author/s, year/s	Therapy	Cancer	Changes of the TCR repertoire	(Refs.)
Robert *et al*, 2014	Anti-CTLA-4	Melanoma	TCR repertoire diversity ↑	(6)
Tumeh *et al*, 2014; Roh *et al*, 2017	Anti-PD-1	Melanoma	TCR repertoire clonality ↑	(44,45)
Kato *et al*, 2021	Anti-PD-1	Renal cell carcinoma	TCR repertoire clonality ↑, TCR repertoire diversity ↓	(46)
Liu *et al*, 2019	Chemotherapy, radiotherapy, tyrosine kinase inhibitor therapy and/or antiangiogenic therapy	Lung cancer	TCR repertoire diversity ↓	(47)
Chen *et al*, 2021	Chemotherapy with bevacizumab or cetuximab	Metastatic colorectal cancer	TCR repertoire diversity ↓	(48)
Sheikh *et al*, 2016	Sipuleucel-T	Prostate cancer	TCR repertoire diversity ↓	(49)

B, Tumor-infiltrating lymphocytes

First author/s, year/s	Therapy	Cancer	Changes of the TCR repertoire	(Refs.)
Akiyoshi *et al*, 2021	Radiotherapy, chemotherapy	Rectal cancer	TCR repertoire diversity ↑	(54)
Ge *et al*, 2023	Cetuximab	Head and neck squamous cell carcinoma	TCR repertoire clonotypes ↑, TCR repertoire diversity ↓	(55)

TCR, T-cell receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein; ↑, increase; ↓, decrease.

6. Application of TCRs in tumor prognosis

There is evidence to suggest that the diversity of the TCR repertoire can be used as a prognostic biomarker of the immune response to tumors. For example, an increased TCR repertoire diversity in PDAC tissue was found to be associated with an improved prognosis (72). However, different findings were reported for muscle-invasive bladder cancer (MIBC); specifically, patients with low TCRβ chain diversity, associated with oligoclonal TIL expansion, had longer recurrence-free survival (73). Although the patients with MIBC had low TCRβ chain diversity, they also exhibited oligoclonal TIL expansion and high number of neoantigens, which improved their prognosis (73). In patients with nasopharyngeal carcinoma, a lower TCR repertoire diversity in tumor tissues than in paired adjacent normal tissues was found to be associated with a poor prognosis. Lower diversity in the tumor may indicate that T cells were hindered from infiltrating or inducing apoptosis in the TME, thus suggesting that the T-cell immune response was insufficient, and that the patient may benefit from checkpoint blockade treatment (74). In a study conducted by Valpione *et al* (75), an increase in the baseline TCR repertoire diversity of tumor-infiltrating T cells was found to be associated with an improved prognosis in patients with various cancers, including breast cancer, melanoma, lung cancer and RCC.

A number of studies have shown that the diversity of the TCR repertoire in peripheral blood is able to predict tumor prognosis. In a study of patients with lung cancer, a lower diversity of the TCR repertoire in the peripheral blood after treatment was found to be associated with a poor prognosis (47). However, in other studies of lung cancer, those patients with a high TCR repertoire diversity in the peripheral blood had an improved prognosis (76-79). In patients with gastrointestinal tumors, a high diversity of the TCR repertoire in the peripheral blood after anti-PD-1 treatment was also found to be associated with a good prognosis (43). Similarly, in metastatic colorectal cancer, a high TCR repertoire diversity in the peripheral blood exhibited an association with a good prognosis (48). Also, in patients with melanoma, a low diversity of the peripheral blood TCR repertoire was associated with a poor prognosis (7). Similar findings have been reported in patients with breast cancer (80), where a low TCR repertoire diversity in the peripheral blood was associated with poor overall survival. In addition, Yan *et al* (81) reported that for patients with esophageal squamous cell carcinoma, a greater TCR repertoire diversity in the peripheral blood was associated with an improved prognosis, while high TCR repertoire clonality was associated with a poor prognosis (Table V).

TCR repertoire diversity has been found to be associated with prognosis in other tumors. In patients with high-grade serous ovarian cancer (HGSOC), the diversity of the TCR repertoire was low in patients who experienced recurrence. Therefore, it was speculated that patients with high TCR repertoire diversity have an improved prognosis (82). In MIBC, basal/squamous-like and stroma-rich subtypes exhibited high TCR richness and diversity, which were associated with a good

Table IV. Associations between high TCR repertoire diversity in peripheral blood and therapeutic effect.

First author/s, year/s	Therapy	Cancer	Therapeutic outcome	(Refs.)
Hopkins *et al*, 2018	Anti-CTLA-4	Advanced pancreatic cancer	Improved	(57)
Postow *et al*, 2015; Arakawa *et al*, 2019	Anti-CTLA-4	Advanced melanoma	Improved	(58,59)
Hopkins *et al*, 2018	Anti-PD-1	Advanced pancreatic cancer	None	(57)
Ji *et al*, 2021	Anti-PD-1	Gastrointestinal tumors	Improved	(43)
Han *et al*, 2020; Dong *et al*, 2021	Anti-PD-1	Non-small cell lung cancer	Improved	(60,61)
Liu *et al*, 2019	Radiotherapy, chemotherapy	Lung cancer	Improved	(47)

TCR, T-cell receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein.

Table V. Associations between TCR repertoire diversity in tumor tissue or peripheral blood and prognosis in different tumors.

A, Tumor tissues

First author, year	TCR repertoire characteristics	Cancer	Prognosis	(Refs.)
Pothuri *et al*, 2024	High TCR diversity	Pancreatic ductal adenocarcinoma	Improved	(72)
Choudhury *et al*, 2016	High TCR diversity	Muscle-invasive bladder cancer	Worse	(73)
Jin *et al*, 2018	Low TCR diversity	Nasopharyngeal carcinoma	Worse	(74)
Valpione *et al*, 2021	TCR diversity in tumor tissues ↑	Breast cancer, melanoma, lung cancer and renal cell carcinoma	Improved	(75)

B, Peripheral blood

First author, year	TCR repertoire characteristics	Cancer	Prognosis	(Refs.)
Liu *et al*, 2019	Diversity of the TCR repertoire ↓	Lung cancer	Worse	(47)
Reuben *et al*, 2020; Chen *et al*, 2022; Wang *et al*, 2021; Abed *et al*, 2023	High TCR diversity	Lung cancer	Improved	(76-79)
Chen *et al*, 2021	High TCR diversity	Metastatic colorectal cancer	Improved	(48)
Yan *et al*, 2022	High TCR diversity	Esophageal squamous cell carcinoma	Improved	(81)
Charles *et al*, 2020	Low TCR diversity	Melanoma	Worse	(7)
Manuel *et al*, 2012	Low TCR diversity	Breast cancer	Worse	(80)
Ji *et al*, 2021	Diversity of the TCR repertoire increased after PD-1 treatment	Gastrointestinal tumors	Improved	(43)

TCR, T-cell receptor; PD-1, programmed cell death protein.

prognosis. However, no changes in TCR richness or diversity were observed for the luminal subtype (83).

In addition to diversity, other characteristics of the TCR repertoire have been found to be prognostically useful. For example, in a study of HCC, similarity of the TCR repertoire between tumor and adjacent normal tissues was indicated to be associated with an improved prognosis (33). However, in patients with NSCLC, high TCR repertoire clonality in the tumor compared with that in adjacent normal tissues was found to be associated with a good prognosis (60,77). A similar finding was reported in a study of HGSOC (84). In patients with advanced RCC, those with elevated TCR repertoire clonality after anti-PD-1 treatment exhibited a good prognosis (46), while in patients with CC, fewer clonal types in the sentinel lymph

Table VI. Associations between other characteristics of the TCR repertoire and prognosis in tumors.

First author/s, year/s	TCR repertoire characteristics	Cancer	Prognosis	(Refs.)
Lin *et al*, 2018	Similar TCR repertoire in tumor and adjacent normal tissues	Hepatocellular carcinoma	Improved	(33)
Han *et al*, 2020; Chen *et al*, 2022	High TCR clonality in tumor tissues	Non-small cell lung cancer	Improved	(60,77)
Lecuelle *et al*, 2021	High TCR clonality in tumor tissues	High-grade serous ovarian cancer	Improved	(84)
Kato *et al*, 2021	TCR repertoire clonality after anti-PD-1 therapy ↑	Renal cell carcinoma	Improved	(46)
Cui *et al*, 2018	TCR repertoire clonality in sentinel lymph nodes ↓	Cervical cancer	Worse	(28)

TCR, T-cell receptor; PD-1, programmed cell death protein; ↑, increase; ↓, decrease.

node TCR repertoire was associated with a poor prognosis (28) (Table VI). Notably, TCR convergence, where T cells share identical TCRs with an identical amino acid sequence yet have different DNA sequences due to codon degeneracy, also has an association with tumor prognosis, with a greater TCR convergence corresponding to an improved prognosis (85). Therefore, TCR repertoire diversity is a promising tumor prognostic marker. However, the relationships between other characteristics of the TCR repertoire and prognosis require verification in studies involving large numbers of patients.

The diversity distribution of TCR repertoires in peripheral blood mononuclear cells (PBMCs) and tumor infiltrates, and their association with prognosis is disparate for tumors of different types. The diversity of a TCR repertoire is determined by the number of different clonotypes (richness) and the relative abundance of these clonotypes (evenness) in a sample (86). All clonotypes derived from clonal expansion carry an identical CDR3 in which the V-region used during V(D)J recombination which depends on subnuclear relocation of the rearranging TCR loci (*tr*), DNA methylation status, recruitment of chromatin remodelling enzymes, histone modification and germline transcription as well as spatial and temporal regulation in different subtypes of cancers, and then influence the number and abundance of clonotypes (87). Additionally, exposure to certain antigens influences the expansion of specific TCR clonotypes. Therefore, the TCR repertoires of different tissues and tumors are dynamic, and dependent on factors such as age and antigen exposure (87). Tumor antigens include tumor-associated and tumor-specific antigens; the latter are only expressed in tumor tissues and activate specific T cells, which conditions TCR repertoire diversity. Peripheral blood TCR diversity is closely associated with the global transcriptomes of peripheral blood and the intratumor microenvironment mainly referred to the number of tumor-specific lymphocytes which spread to peripheral blood (88). Additionally, peripheral blood TCR diversity decreases while the clonality increases with age (89). This may explain the discrepancy between TCR repertoires in PBMCs and tumor infiltrates.

7. Conclusions and prospects

The importance of the TCR repertoire in tumor immunity has become increasingly apparent. In the present review, TCR structures are summarized. TCR repertoire characteristics are potentially useful as tools for the diagnosis of tumors, the enhancement of tumor immunity and the prediction of tumor prognosis. The TCR repertoire holds promise as a biomarker, and patients are expected to benefit from research into this repertoire. However, compared with the immunohistochemical detection of PD-Ls and targeted sequencing of liquid biopsies, which allows for the detection of tumor-derived DNA or circulating tumor cells that are present in the bloodstream or other bodily fluids, TCR repertoire analysis is complex and expensive. It comprises multiple steps, including the preparation of blood or tissue samples, TCR sequencing, data analysis and interpretation, which require professional and technical personnel, high-throughput sequencing platforms and bioinformatics tools. Moreover, the volume of data generated by TCR sequencing is substantial, and the identification of specific TCRs for cancer therapy is time consuming (19). Novel prediction models have been developed to identify epitope-specific TCRs (90). In addition, more sensitive and cost-effective sequencing tools have been developed, including characterizing TCR repertoires (91), spatially resolved TCR sequencing (92), and formalin-fixed paraffin-embedded-suitable unique molecular identifier-based-TCR sequencing (93). Tools such as these are expected to further utilize and maximize the value of TCR repertoire analysis. Although TCR analysis indicators, such as Shannon entropy and clonality, can help in understanding the relationships between TCR clonality and solid tumors, the associations between TCR repertoires and clinical benefits require further investigation. Nevertheless, the TCR repertoire can be used as a tumor immune biomarker, with potential clinical significance in the prediction of patient prognosis and the monitoring of therapeutic efficacy. In the future, more advanced sequencing, database construction techniques and comprehensive analysis algorithms are likely to be developed to further simplify and clarify the evaluation and comparison of TCR repertoires, reduce the cost of TCR repertoire analysis, and allow more people to benefit.

In addition, despite the extreme diversity of TCR chain pairs, the specific antigens recognized by the TCRs of γδ T cells remain largely unknown (94). Therefore, more research into γδ T cells is necessary. Studies have shown that γδ T cells play an important role in tumor immunotherapy (95-98) and can directly attack tumor cells without relying on MHC for antigen presentation. Therefore, in the future, in addition to further study of αβ T cells, more attention should be focused on γδT cells. The assessment of these cells may promote the further development of tumor immunotherapy.

A recent review by Aran *et al* (86) described the assembly and structure of TCRs, the sequencing and analysis of TCRs, and the prognostic and predictive values of the TCR repertoire as a biomarker in the treatment of cancer using ICIs. In the present study, the potential use of various TCR repertoires as biomarkers for tumor diagnosis are presented, and some characteristic changes in the TCR diversity of peripheral blood and tumor tissues are discussed. Furthermore, the limitations of the TCR repertoire as a biomarker are considered, such as the complexity and high cost of testing; the reasons and common clinical countermeasures are also briefly discussed. The present review also provides updates on recent research advancements in the development and application of TCR repertoires in tumors including thyroid cancer (98), MIBC (83), HGSOC (82), advanced or metastatic NSCLC (79), PDAC (72) and PSCC (36), and their associations with tumor immunotherapy, diagnosis and prognosis.

Acknowledgements

Not applicable.

Funding

This study was supported by a fund from the Science and Technology Cooperation and Exchange Special Project of Shanxi Province (grant no. 202104041101012).

Availability of data and materials

Not applicable.

Authors' contributions

ALH drafted the manuscript and was responsible for drawing the figure and preparing the tables. YZH and YY were responsible for the acquisition, analysis and interpretation of data. GPZ, HLW and MP reviewed and revised the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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