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Review

Application of immune repertoire sequencing in cancer immunotherapy

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ABSTRACT

With the prominent breakthrough in the field of tumor immunology, diverse cancer immunotherapies have attracted great attention in the last decade. The immune checkpoint inhibitors, adoptive cell therapies, and therapeutic cancer vaccines have already achieved impressive clinical success. However, the fact that only a small subset of patients with specific tumor types can benefit from these treatments limits the application of cancer immunotherapy. To seek out the molecular mechanisms behind this challenge and to select cancer precision medicine for different individuals, researchers apply the immune repertoire sequencing (IRS) to evaluate genetic responses of each patient to current immunotherapies. This review summarizes the technical advances and recent applications of IRS in cancer immunotherapy, indicates the limitations of this technique, and predicts future perspectives both in basic studies and clinical trials.

1. Introduction

The human adaptive immune system has evolved many mechanisms to protect tissues and organs from being damaged by foreign pathogens or cancer cells [1]. Notably, the B and T lymphocytes are equipped with antigen receptors to bind different antigens, thus activating the immune system to attack specific enemies with efficiency and accuracy. Human immune repertoire generally refers to the total collection of T cell receptors (TCRs) and B cell receptors (BCRs), which are generated with enormous diversity through somatic rearrangement and hypermutation. The complementarity determining region 3 (CDR3) of TCR and BCR is the most variable gene region that determines the specificity of antigen recognition [2]. The diversity of CDR3 mainly depends on the recombination of two or three gene segments including variable (V), diversity (D), and joining (J), as well as gene insertion/deletion. Additionally, matured B cells acquired somatic hypermutation to make the diversity of BCR more complicated, as Fig. 1 illustrated. Due to the astronomical number of potential CDR3 rearrangements [3], traditional techniques based on proteomics are no longer suitable for large-scale analysis of immune repertoire [4,5]. However, the tremendous technical improvement of next-generation sequencing (NGS) enables high throughput analysis of immune repertoire both at mRNA and DNA levels, rendering the IRS more affordable for wide applications.

As a great number of clinical achievements have been made during the past years, cancer immunotherapy ranks as one of the most popular cancer therapies. The approvals of Yervoy (ipilimumab), Opdivo (nivolumab), and Kymriah (tisagenlecleucel) by the US Food and Drug Administration (FDA) verified the effectiveness and superiority of immunotherapeutic medicines in clinical trials. After the treatment of immunotherapy, tumor regressions and prolonged overall survival rates were observed in patients with metastatic cancers, and some individuals even achieved complete remission [6]. Despite the encouraging results, certain limitations of immunotherapy emerge at the same time. In general, low response rate and drug resistance are major factors that hinder the development of wide-spectrum cancer immunotherapy. To better understand the molecular background of tumor immunology and design personalized immunotherapy, the immune repertoire analysis has been applied in recent cancer research. With the immune repertoire data collected before and after cancer treatment, one can evaluate the state of tumor microenvironment and predict the effect of the immunotherapy [7]. Moreover, the combination of IRS and other molecular methods such as recombinant expression cloning may identify potential targets for therapeutic cancer vaccines [8,9]. In this review, we introduced the latest technology of IRS and the current application of this technology in tumor monitoring and cancer immunotherapy. Notwithstanding the limited examples of clinical application so far, immune repertoire holds good promise to be a useful biomarker for cancer immunotherapy in the future.

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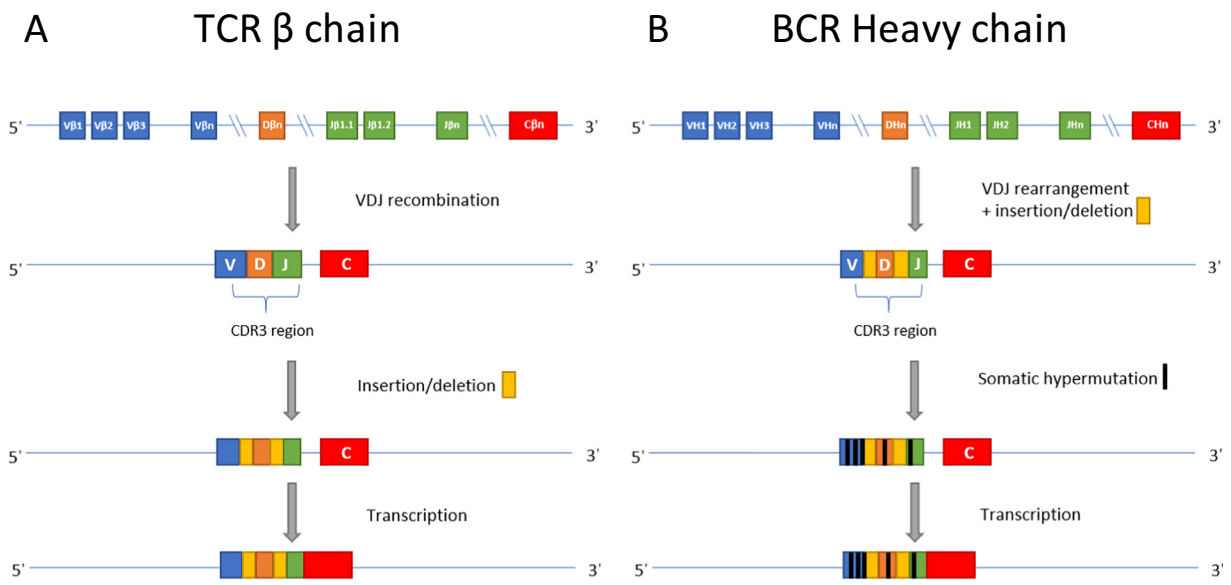

Fig. 1. Gene recombination and transcription of TCR and BCR.
The V, D, J gene segments of TCR and BCR undergo somatic rearrangement and gene insertion/deletion before transcription, generating highly variable CDR3 regions which constitute diverse lymphocytic receptors that target different antigens. A) CDR3 rearrangement of TCR β chain; B) CDR3 rearrangement of BCR heavy chain.

2. Methodologies for immune repertoire analysis

Thanks to the technical development of NGS, nowadays the analysis of immune repertoire is neither too difficult nor expensive as it was ten years ago. According to a protocol published in 2017, the cost for preparation of a TCR library can be as low as £13.7 [10]. The price for sequencing has also been reduced to a large extent. Besides the cost, samples for generating immune repertoire are flexible and easy to acquire. DNA or RNA samples extracted from peripheral blood mononuclear cells (PBMCs), formalin-fixed paraffin-embedded (FFPE) tissues or selected cell populations are commonly used, depending on different purposes of research. Currently, the basic process of IRS mainly includes four sections: 1) cell isolation or tissue collection, 2) extraction and purification of DNA or RNA, 3) target amplification and library construction, 4) sequencing [11]. The general workflow for IRS is displayed in Fig. 2. Since the V segment of immune repertoire is highly variable, two NGS-based amplification methods are predominately applied to decode the CDR3 sequences of both TCR and BCR. One is called multiplex PCR, in which multiplex primer panels are applied for amplifying the diverse V and J sequences or adjacent constant (C) sequences of B/T cell receptors. The other one is called rapid amplification of cDNA ends (5′RACE) PCR, in which the RNA of lymphocytes is reverse transcribed with a gene-specific primer targeting known sequence of 3′end, while the 5′end of unknown sequence is amplified with an attached synthetic oligonucleotide. Both methods were proved stable and reliable in different studies related to immune repertoire [12–16]. However, a comparative study indicated that multiplex PCR is inclined to amplification biases, whereas 5′RACE PCR generates limited valid data [17]. To reduce these disadvantages, new approaches derived from multiplex PCR and 5′RACE PCR are developed constantly. Eberlein et al. introduced an Anchored Multiplex PCR (AMP) technology, which improved the sensitivity of traditional multiplex PCR and decreased amplification bias with molecular barcodes (MBCs) [18,19]. Oakes et al. used T4 RNA ligase to add unique molecular identifier (UMI)-labeled oligos to V-region end of cDNA instead of the template switch transcriptase, significantly reducing the cost and increasing the flexibility of 5′RACE PCR [10]. Moreover, a TCR ligation-anchored-magnetically captured PCR (TCR-LA-MC PCR) enabled deep sequencing of TCR transcripts and enhanced the proportion of valid data by using gene-specific biotinylated primers [20]. Although not frequently used, the target enrichment or RNA-capture method is also applied for IRS. However, the required sequencing depth for RNA-capture method may be 35–50× higher to reach the same level of target reads that generated by other methods [21].

Artificial error brought from PCR amplification is the most significant problem for NGS. As for IRS, amplification errors also lead to bias in final data, and these errors should be avoided to the maximum extent. Therefore, researchers designed the unique molecular identifier to mark each original PCR template, whose amplicons are clustered and mapped, eliminating the majority of PCR and sequencing errors [22,23]. Due to the high efficiency and tremendous information generated by IRS, scientists are no longer satisfied with bulk sequencing of cell populations but tend to focus on the TCR/BCR of a single cell. Single-cell sequencing can tell more valuable stories, especially in cancer evolution and tumor immunity [24]. Technology of single-cell sequencing allows for simultaneous analysis of transcriptional profile and TCR/BCR sequences [25,26]. Using the bioinformatic tools, researchers can pair the sequences of different chains of TCR (αβ, γδ chains) or BCR (heavy and light chains) from the single cell, acquiring integrated information. This improvement promotes the application of immune repertoire analysis in vaccine development and minimal residual disease (MRD) detection [27,28]. However, various applications of IRS call for different bioinformatic tools for data analysis. The international ImMunoGeneTics information system (IMGT) plays important roles in the mapping of TCR and BCR repertoires [29]. It is a useful database for inquiring information about specific V, D, J, C genes of TCR and BCR. Besides, many public bioinformatic tools for immune repertoire analysis have been released, including MiXCR, IMonitor, IMPre, tcR, ClonoCalc and ClonoPlot [30–36]. For the guideline of data analysis, Gur and Steven summarized the analytical procedure for B cell repertoire sequencing [37]. In spite of these public packages, algorithms, and protocols created for immune repertoire profiling, the fast development of technology and extensive applications of immune repertoire continually make space for new analytical tools.

3. Immune repertoire as a biomarker for cancer screening and monitoring

The immune repertoire, as a particular biomarker reflecting the clonal lymphocyte populations and receptor diversity, was found

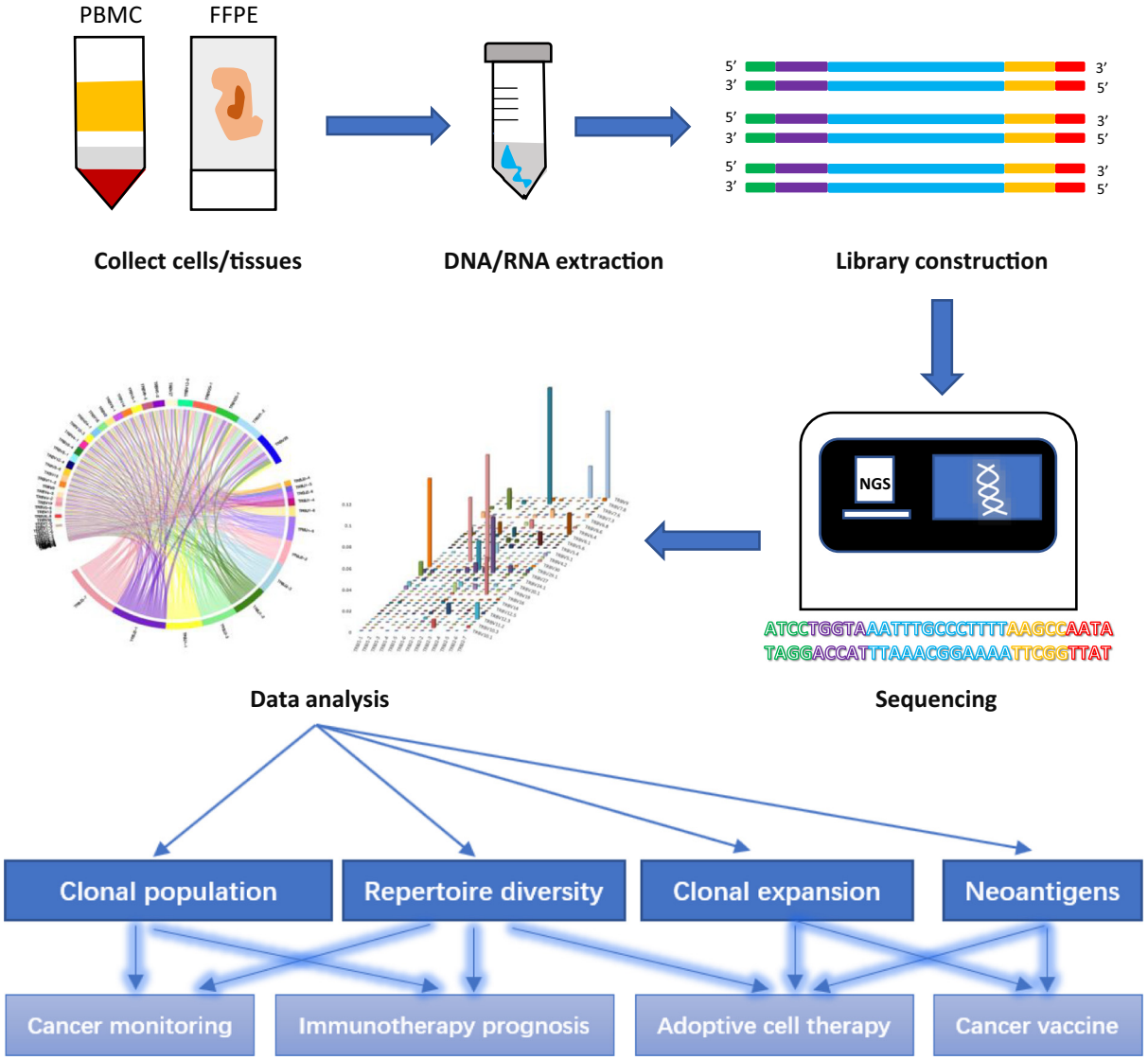

Fig. 2. General workflow for IRS and downstream application.
Samples including lymphocytes and tissues can be used for immune repertoire analysis. DNA or RNA are extracted and amplified with specific primers to generate TCR/BCR library. Immune repertoire data are produced by NGS and analyzed with bioinformatic tools based on different research objectives. Immune repertoire analysis is widely applicable in many fields of immunotherapy, certain indexes (dark blue square) are valuable for different applications (light blue square). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

clinically valuable in diagnosing and monitoring blood cancers [1]. Mining effective information of immune repertoire is a key to explore the molecular mechanism of tumor immunology, which is important for searching cancer antigens and developing new immunotherapies [38]. Interestingly, the mammalian immune system has dual effects on tumor. On one hand, suppressed immune system favors the escape of tumor cells from the original location, leading to uncontrolled metastasis. On the other hand, enhanced immune system generates overgrowing lymphocytes, inducing hematopoietic malignancies. The balance between immune responses and tumor growth is delicate. Therefore, monitoring immunological changes under different tumor microenvironment is essential for tracking lymphoma and leukemia. Since cancer cells proliferate endlessly, malignant clones generally take up the majority of immune repertoire, and the frequency of specific gene sequences exceeds others. As a result, significant difference of B cell repertoire was observed between the healthy donors and patients with leukemia and lymphomas, providing an implication for diagnosis of blood malignancies [39,40]. Due to the development of high-throughput sequencing, the minimal residue disease in leukemia can be detected comprehensively with TCR and BCR sequencing, which demonstrated much higher sensitivity compared with flow cytometry and other approaches [28,41]. However, it is wise to be prudent with these data because high sensitive method may increase the number of false positive results regardless of clinical significance. In addition to blood cancer and lymphomas [42], IRS can also be used for predicting clinical prognosis of many other cancers, such as gastric cancer and hepatocellular carcinoma [43–45].

Researchers have made great efforts to explore the molecular mechanisms of cancer. Mutations of tumor-associated genes were proven responsible for cancer development and drug resistance [46–49]. Other than specific gene mutations, the heterogeneity of tumor and immunosuppressive mechanisms together increase the complexity of tumorigenesis and the risk of cancer therapy [50]. Faced with these challenges, the immune system excavates smart ways to expose the disguised cancer cells and eliminate them. Tumor infiltrating lymphocytes (TILs) play important roles in this process. By characterizing the immune repertoire of the TILs in different locations, researchers can distinguish the T cell population reshaped by tumor specific antigens

[51,52]. In addition, the intratumor heterogeneity can also be elucidated by comparing the immune repertoire of TILs with that of healthy adjacent tissues [53,54]. It was reported that the heterogeneity of neoantigens was correlated with the possibility of cancer relapse, and the neoantigen landscape of localized tumor could be predicted from the diversity of TILs immune repertoire [55]. Notably, tumor neoantigens are also critical targets of cancer immunotherapy. The surveillance of tumor microenvironment and the discovery of neoantigens promote the development of efficient cancer immunotherapy for different cancers. Therefore, IRS may be a useful technology for developing new cancer immunotherapy. Moreover, this technology also helps evaluate the responses and effects of various immunotherapies as discussed next.

4. Application of immune repertoire analysis in cancer immunotherapy

4.1. Immune checkpoint therapy

Tumor cells have developed many tactics to evade surveillance and attack of the immune system, including decreased expression of tumor antigen, promotion of tumor cell growth, and inhibition of lymphocytic functions [56]. In some cases, the immune system fails to eliminate the altered tumor cells but inversely protect them by establishing a privileged tumor microenvironment [57]. To break these tactics, researchers have developed several therapeutic strategies that reinforce the suppressed immunity. Blockade of immune checkpoints is one of the remarkable immunotherapies targeting multiple tumor types, especially for melanoma [58]. So far, this immune checkpoint therapy mainly focuses on three target molecules: CLTA-4, PD-1 and PD-L1, which are involved in the immunosuppressive mechanism. Antibodies against these targets can activate the restrained T cell functions, demonstrating significant clinical responses against cancer [59]. Clinical trials have shown that immune checkpoint therapy improves the overall survival and long-term safety of cancer patients, induces fewer toxic effects and metastases in cancer patients compared with traditional cancer treatments such as radiation therapy and chemotherapy [60–62]. However, different patients might have contrary clinical outcomes due to the diversity of immune repertoire and lymphocytic population. It is critical to select the right patients who are likely to benefit from the immunotherapy and suffer fewer side effects [63].

Although several biomarkers are correlated with the outcome of immune checkpoint therapy, including the expression of PD-L1 [64], tumor mutational load [65], tumor-infiltrating lymphocytes [66], mismatch-repair defects [67], and immune gene signatures [68], none of these biomarkers indicates the dynamic changes of the immune cell population before and after the immunotherapy. The immune repertoire seems to be a promising candidate that reflects the direct cell-mediated immune responses caused by immunotherapy. It is reported that, increased diversity (clonal richness and Shannon diversity) of peripheral TCR repertoire was identified in patients after the CTLA-4 blockade therapy, while no significant change was observed in healthy donors [69]. This increase was in association with better tumor response and higher toxicity in patients receiving CTLA-4 inhibiting antibody treatment. However, no specific clonal expansion was found correlated with outcomes of anti-CTLA-4 immunotherapy. Another study related to anti-PD-1 (nivolumab) immunotherapy also indicated that, richness and evenness of TCR repertoire were associated with clinical benefits in patients receiving nivolumab treatment [70]. Interestingly, patients who received anti-CTLA-4 (ipilimumab) therapy before nivolumab were more likely to benefit from the latter treatment if their TCR repertoire showed higher richness. Whereas, patients who benefited from nivolumab therapy alone had a TCR repertoire of lower evenness compared with those non-responders. The shifts in TCR repertoire might result from reinforced antigen recognition, which was inhibited by the binding of PD-1 and PD-L1 receptors between T cells and tumor cells before immune checkpoint blockade. Integrative analysis of tumor mutations and TCR repertoire revealed that the expansion of specific T cell clones was linked to the decrease of tumor neoantigens on therapy, indicating the TCR repertoire dynamics might predict whether patients were responsive to anti-PD-1 immunotherapy [70]. Similar conclusion was verified with immunohistochemistry methods, indicating the reliability of immune profiling results generated by IRS [71]. Following the arise of immunotherapy, many researchers began to find a combinatory treatment incorporating both traditional therapy and immunotherapy for cancer patients [59]. Data showed that radiation enriched the TCR diversity of TILs, while anti-CTLA-4 therapy increased T cell clones. Together, the abundant TCR repertoire promotes tumor rejection [72]. The addition of anti-PD-L1 therapy can further induce the T cell expansion by reactivating exhausted T cells after the anti-CTLA-4 treatment [73]. Although immune repertoire analysis may be valuable for predicting or evaluating the effect of immune checkpoint therapy, other potential biomarkers such as the expression of PD-1, PD-L1 should also be taken into consideration simultaneously to make the evaluation more comprehensive [74].

Apart from the uncertainty of therapeutic efficacy, the immune-related adverse effect is another challenge for immune checkpoint therapy. Many patients who received anti-CTLA-4 or anti-PD-1 therapies developed mild or moderate side effects which are usually reversible, such as colitis, enteritis, hepatitis and skin inflammation. Since these symptoms are resulted from active immune responses, some researchers linked the immune toxicity with therapeutic efficacy [75,76]. Severe cases were also reported about the adverse events of neurological disorders and cardiac diseases, which might be life threatening. Thus, prediction and assessment of toxicity are critical for the development of immunotherapy. Clinical studies suggested that cancer patients were more likely to develop immune-related toxicities if their T cell diversity increased early after the anti-CTLA-4 therapy or if clonal expansion of CD8 T cells was detected in their peripheral blood post-therapy [77,78]. Additionally, decreased circulating B cells as well as enriched plasmablasts and $CD21^{lo}$B cell subset were correlated with immune-related adverse events in patients treated with anti-CTLA-4 and anti-PD-L1 combination therapy [79,80]. These findings give us implications that changes of immune repertoire may be potential predictive biomarkers for adverse effects after the immune checkpoint inhibitor therapy.

4.2. Adoptive cell therapy

Adoptive cell therapy (ACT) is often called a “living” immunotherapy. Functional T cells are specifically selected or modified in vitro and are infused back to the blood of patients for regulating cancer regression [81]. Tumor infiltrating lymphocytes was first used in ACT to treat metastatic melanoma [82]. Afterwards, two types of genetically engineered T cells, harboring specific T cell receptors (TCRs) and chimeric antigen receptors (CARs) respectively, were invented for treating other human cancers. The receptors target tumor-specific antigens and they can guide the engineered T cells to infiltrate tumor. Patients with hematologic malignancies have obtained positive clinical results after CAR-T cell treatment. However, effective ACT treatments for epithelial solid cancers are still in development. Due to the intratumor heterogeneity, ACT only induces limited responses for solid tumors in certain patients. And the toxicity for normal tissues, which have similar antigens as targets of engineered T cells, is also a big problem in ACT treatment.

To select accurate and specific T cells that destroy tumor but not normal tissues, a proper biomarker is needed for monitoring both pharmacological responses and immunogenomics during the ACT treatment. Many biomarkers have been tested in T cell therapy clinical trials for various purposes, including surface and intracellular markers, transcriptional profiling, cytokine profiling, humoral immune responses and so on [83,84]. Among these biomarkers, TCR repertoire stands out due to the high sensitivity of IRS. Researchers have succeeded in

generating antigen-specific T cells that express rearranged endogenous TCR from induced pluripotent stem cells [85,86]. These engineered T cells are then infused to patients as cell-based immunotherapy. Given the unique characteristics of each T cell population, TCR repertoire sequencing can be used to track the clonal expansion and persistence of the infused T cells [87]. In addition, TCR repertoire sequencing of TILs provides significant information about intratumor heterogeneity and clonal distribution, serving as a promising biomarker for cancer prognosis [88]. Numerous studies investigated the heterogeneity of TILs with TCR sequencing, and different diversity of TCR repertoire was observed between TILs and normal adjacent tissues from patients with colorectal cancer [51]. However, the TCR repertoire seems spatially homogeneous within the tumor tissues in ovarian cancer patients, while TCR repertoire of peripheral blood is different from that of tissues [89]. Based on the TCR changes of TILs before and after therapy, T cell responses and efficacy of immunotherapy may be evaluated [90].

Adoptive cell therapy has achieved remarkable progress in curing hematological malignancies. Engineered TCR-T and CAR-T cells are widely applied in this therapy, demonstrating anti-leukemia reactivity which leads to cancer regression in patients [6,91–93]. The TCR repertoire of patients with B-cell chronic lymphocytic leukemia (B-CLL) showed different patterns of TCR β chain variable gene usage and clonal population compared with normal population [94]. While after immunotherapy, the T cell repertoire was reconstituted, and the changed TCR patterns might predict disease progression [95]. Improved diversity of T cell repertoire was observed after ACT not only in patients with leukemia but also in patients with solid tumor such as neuroblastoma [96,97], suggesting that the detection of TCR repertoire may serve as a universal diagnostic approach in ACT. Since these cell therapies require active T cells, T cell senescence and exhaustion after infusion remain a challenge for ACT [98]. Therefore, T cell viability and robustness should also be considered when designing CAR or TCR constructs. As TCR activation has different influence on the exhaustion of CD8 and CD4 CAR-T cells [99], selecting correct T cells for TCR repertoire sequencing is a prerequisite for the successful application in clinical tests.

While T cells attract wide attention for playing key roles in anti-tumor immunity, the function of B cells in tumor microenvironment is probably overlooked [100]. Actually, enriched tumor-infiltrating B (TIL-B) cells are also associated with the favorable prognosis as the companion of activated T cells, especially for patients with nonmetastatic tumors [101]. Conversely, the risk of relapse was higher for patients whose pulmonary tumor harbors fewer plasma B cells [102]. Genetic analysis indicated that the expression level of BCR gene segments was correlated with overall survival and progression-free survival of patients with basal-like and HER2-enriched breast cancer as well as immunoreactive ovarian cancer [103]. Related studies showed different BCR repertoire characteristics of TIL-B cells in invasive ductal carcinoma and medullary breast carcinoma, demonstrating uneven B cell immune responses and infiltrating status in different breast cancers [104,105]. The tertiary lymphoid structures, consisted of various immune cells, widely exist in many inflamed tissues as well as tumor. These structures were found associated with positive immune responses and clinical benefits in lung cancer [106,107]. The role of B cells in the tertiary lymphoid structure is closely related to anti-tumor activities, based on the detection of antigen-driven B cell maturation [107]. BCR sequencing is a useful tool for monitoring the molecular features of B cell differentiation that resulted from the recognition of tumor antigens, by analyzing the characteristics of clonal amplification, somatic hypermutation and class switching [108]. Thus, B cell repertoire may also serve as a potential biomarker for the prognosis of cancer treatments. Furthermore, as antigen-presenting cells, TIL-B cells may activate and sustain the T cell responses, promoting better clinical outcomes in cancer patients [109]. From this perspective, new adoptive cell therapy can be developed by combination of both T cell and B cell products or using B cell products as an adjuvant [110,111]. Therefore, parallel sequencing of TCR and BCR may be more informative for the prognosis of adoptive cell therapy.

4.3. Vaccine therapy

Vaccine is regarded as a useful tool for preventing diseases. Traditional vaccines are developed on pathogen-associated antigens, which are distinguished from the self-antigens of the host. However, the cancer therapeutic vaccine is very complicated. Tumor specific antigens are quite rare, because many antigens within tumor are also expressed on normal tissues. Vaccines targeting these unspecific tumor antigens may lead to unexpected immune responses and toxicity. Thus, mutated antigens which are unique to cancer or cancer-related viruses should be considered as prior targets for vaccine development [112]. Not only have preventive vaccines been proved successful, such as hepatitis B virus (HBV) and human papillomavirus (HPV) vaccine, but also therapeutic vaccines aiming at tumor neoantigens have indicated clinical benefits. Prolonged survival was observed as the main result of therapeutic cancer vaccines in many clinical studies, although the effect on objective durable regression was somehow compromised compared with other immunotherapies [113]. Taking a step back, since most of tumor antigens are similar to self-antigens while the expression levels of both are quite different, vaccines targeting the shared antigens also play an important role in cancer immunotherapy. However, these vaccines should be carefully modulated to circumvent immunological tolerance mechanisms without causing over immune responses. Considerable approaches have been used for monitoring the immunization after inoculation of cancer therapeutic vaccines, including enzymatic assays, serological methods and immune repertoire analysis [112,113].

Immune repertoire is a multi-functional indicator that helps evaluate the effects of vaccines, select vaccine targets and predict cancer prognosis. On one hand, effective cancer therapeutic vaccines can induce immune responses, reshaping the immune repertoire with significant patterns such as specific clonal expansion, changed TCR/BCR diversity and evenness [15,114]. By analyzing the change of immune repertoire pre- and on-therapy, one can estimate whether the vaccine is effective to the inoculator. On the other hand, TCRs that recognize the same antigen that have conserved CDR3 sequences, and the diversity of TCR repertoire is affected by the antigen evolution [115,116]. With TCR repertoire sequencing technology, researchers can discover new antigens or optimize the pre-existing vaccines. Notably, TCRs of the cytotoxic T lymphocytes (CTLs) play critical roles in antigen recognition, including viral antigens and tumor associated antigens. Investigation of the repertoire of CTLs may give informative implications for antigen selection, T cell activation and exhaustion during the process of antigen recognition, promoting the development of cancer therapeutic vaccines [117,118]. In addition, BCR repertoire analysis has also been applied in tracking memory B cell recall and B cell response after vaccination as well as antibody selection [9,15,27]. By BCR sequencing, researchers can either identify the tumor-reactive B cells or distinguish shared antibodies that target public tumor antigens from those targeting neoantigens in specific cancer [119,120]. Both ways are useful for the development of anti-tumor antibodies. Moreover, IRS clearly reflects B/T cell responses and clonal expansion after the vaccination, making the evaluation of different delivery methods for vaccine more visual and convenient. Since proper delivery method is difficult to optimize during the development of cancer therapeutic vaccine [121], application of immune repertoire analysis may promote the selection or even discovery of innovative delivery methods.

5. Conclusion

Although new technologies accelerate the development of IRS, there are certain limitations that withdraw wide application of this molecular detecting approach. First, the present methods for IRS have objective disadvantages, which may lead to biased results. Data generated by

various methods coupled with different analytical tools may not be uniformly reliable and robust. For this problem, the Adaptive Immune Receptor Repertoire (AIRR) Community is developing standards for generating reproducible and shareable data of IRS, including protocols, data repositories and analytical tools [122–124]. However, gold standard for IRS that can be applied to every laboratory is still scarce so far. Second, samples collected from different materials such as peripheral blood lymphocytes or tissues have different TCR repertoires, which may result in inconsistent consequences. The randomness of re-sampling and instability of sample preservation also add variations to the IRS results, even for the same sample. Thus, it is necessary to consider the sample differences and sampling reproducibility before we apply IRS for cancer diagnosis and evaluation of immunotherapy. Third, although abnormal evenness and skewed diversity of immune repertoire may indicate unhealthy status of immune system, lacking of a solid cutoff value remains the major barrier for clinical application of IRS. Fourth, despite many bioinformatic tools of immune repertoire analysis that have been developed so far, the reliability of these tools is difficult to evaluate as no gold standard has been verified for immune repertoire. Hopefully, along with the accumulation of clinical samples devoted to immune repertoire studies, more regulations and quality control methods for immune repertoire detection would be established.

In the foreseeable future, IRS holds great possibilities to contribute to basic biological studies as well as clinical applications. In the aspect of basic study, IRS can help us understand the regulation of immune responses during cancer development. Tumor infiltrating cells are important biomarkers for cancer prognosis and tumor metastasis. By comparing the TCR repertoire of tumor infiltrating cells under different spatial and temporal conditions, researchers may find invaluable neoantigens and pivotal changes in tumor microenvironment, thus accelerating the discovery of new cancer therapy. As for the application in clinical trials, IRS may serve as a promising approach for the dual evaluation of immunotherapy, from the aspects of therapeutic effect and adverse effect. In addition, immune repertoire is a good biomarker for tracking the infused lymphocytes in adoptive cell therapy, including the clonal expansion, exhaustion and apoptosis. Moreover, TCR or BCR repertoire sequencing can be applied in the assessment of therapeutic cancer vaccines, implicating the efficacy of different vaccines and drug resistance. With the development of theoretical and practical knowledge about tumor immunology, we should nourish hope for making cancer a more controllable disease sooner or later.

Declaration of Competing Interest

None.

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