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Review

Molecular Mechanisms and Countermeasures of Immunotherapy Resistance in Malignant Tumor

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Abstract

Tumor immunotherapy inhibits the proliferation and invasion of tumor cells by inducing or enhancing anti-tumor immune responses in active or passive ways. It is the fourth therapeutic method with efficiency and safety in addition to surgery, radiotherapy and chemotherapy. At present, anti-tumor immune related clinical trials have made promising achievements in prolonging progression free survival and overall survival, therefore, FDA approved a variety of immune checkpoint blockers (ICBs) such as nivolumab, pembrolizumab, ipilimumab. However, primary or acquired resistance results in massive perplexity to oncologist and patients. In order to bring further clinical benefit to tumor patients, study on mechanisms of immunotherapy resistance is extremely urgent. This review summarizes related mechanisms of tumor immunotherapy resistance, including MITF suppression, Ezh2 upregulation, TIM-3 upregulation, microRNA-driven deregulation of cytokine expression and et al. Genetic mutations such as PTEN loss, JAK1/2 loss-of-function mutations and Cbl-b deficiency are also involved. Moreover, we have discussed feasible countermeasures, for instance, combining ICBs with PRRs agonists, ARNAX, CpG oligonucleotide, oncolytic peptide LTX-315 and indoleamine 2, 3-dioxygenase inhibitors, respectively. Other methods include combined ICBs with radiotherapy, combined ICBs with blockade of PI3K-AKT, TIM-3 pathway; blockade of Fcγ receptors before anti-PD-1 monoclonal antibodies administration and modulation of the gut microbiome, et al. Mechanisms and countermeasures of immunotherapy resistance still requires further exploration, in expectation to provide novel ideals and basis for tolerant patients.

Key words: immunotherapy, resistance, checkpoint, combination therapy

Introduction

Tumor immunotherapy inhibits the proliferation and invasion of tumor cells by inducing or enhancing anti-tumor immune responses in active or passive ways. It is the fourth therapeutic method with efficiency and safety in addition to surgery, radiotherapy and chemotherapy. It is mainly divided into passive immunotherapy and active immunotherapy. Passive immunotherapy includes monoclonal antibody (mAb) and antibody-drug conjugate while active immunotherapy contains cancer vaccine and chimeric antigen receptor T-cell immunotherapy (CAR-T) [1].

Currently, many clinical trials related to immunotherapy have made remarkable achievements. Phase II - IV clinical trials show that immune checkpoint blockers (ICBs) significantly prolong progression free survival, overall survival in melanoma[2,3,4] non-small cell lung cancer (NSCLC)[5,6] head and neck squamous cell carcinoma (HNSCC)[7], liver cancer[8], colorectal cancer[9], urothelium carcinoma[10], renal cell carcinoma[11], classical Hodgkin lymphoma[12] and gastric cancer[13]. Hence, FDA has approved a variety of ICBs for clinical therapy in multiple tumors. Ipilimumab has been approved for

melanoma, while nivolumab and pembrolizumab have been approved for not only melanoma but also other malignant tumors. Meta-analysis showed that the validity and safety of PD-1/PD-L1 inhibitors are prior in NSCLC patients when compared to docetaxel-based chemotherapy, especially in prolonging progression free survival and overall survival[14].

Resistance occurrence in clinical immunotherapy

Clinicians and patients are high with the hope of curing cancer, for the immunotherapy superiority. However, some patients show innate resistance while other patients develop acquired resistance after several courses of immunotherapy. Schadendorf, et al summarized several clinical trials (ID# CA184-007[15], CA184-008[16] and CA184-022[17]) and demonstrated that 25% of 88 metastatic melanoma patients had primary resistance[18]. According to pooled analysis in 655 advanced metastatic melanoma patients, the twelve-month progression-free survival rate was 35% and the median overall survival was 23 months, yet 25% patients appeared recurrence and resistance at 21 months[19]. Ipilimumab, an anti-CTLA-4 mAb, has better effect on survival improvement in BRAF V600E-negative malignant melanoma compared to radiotherapy and chemotherapy. Despite the significant prolonged progression-free survival and overall survival, the majority of patients developed resistance within one year[20]. Hence, primary and acquired resistance results in massive perplexity to oncologist and patients, making study on mechanisms of immunotherapy resistance extremely urgent in order to bring better clinical benefit to tumor patients.

Optimal management of immunotherapy is needed to avoid resistance. There are two major challenges in management optimization: identification of sensitive patients and exploration of immunotherapy resistance mechanism. Gene mutation detection partly identifies primary and acquired resistance[21,22]. Resistance to ICBs differentiates in patients and tumors. Analyzing mechanisms of tumor immunologic escape in different levels conduces to formulating corresponding countermeasures[23]. This review summarizes related mechanisms of immunotherapy resistance and feasible countermeasures in expectation to improve prognosis of immunotherapy resistant patients.

Occurrence of resistance to immunotherapy

Research on anti-PD-1 therapy showed that key genetic mutations, antigen presentation alternations and loss of the IFN-γ signaling pathway related genes can effectively block the ongoing therapeutic immune response[24]. Immunogenicity diversity caused by genetic mutations is the major determinant of immunotherapy resistance. The efficacy of immunotherapy is determined by gene expression profile differential[25]. Primary or acquired resistance to tumor immunotherapy arises in multiple ways, including microphthalmia-associated transcription factor (MITF) suppression, increased enhancer of zeste homolog 2 (Ezh2) expression, microRNA-driven deregulation of cytokine expression, T cell immunoglobulin mucin-3 (TIM-3) upregulation and Wnt/β-catenin signaling pathway activation. Genetic mutations such as phosphatase and tensin homolog deleted on chromosome ten (PTEN) loss, Janus Kinase 1/2 (JAK1/2) loss-of-function mutations and Casitas B-lineage lymphoma-b (Cbl-b) deficiency are also involved (Figure 1). Recent study has also reported that truncating mutation of β-2-microglobulin (B2M) is responsible for antigen presentation, resulting in resistance to anti-CTLA-4 and anti-PD-1 tumor therapy[26].

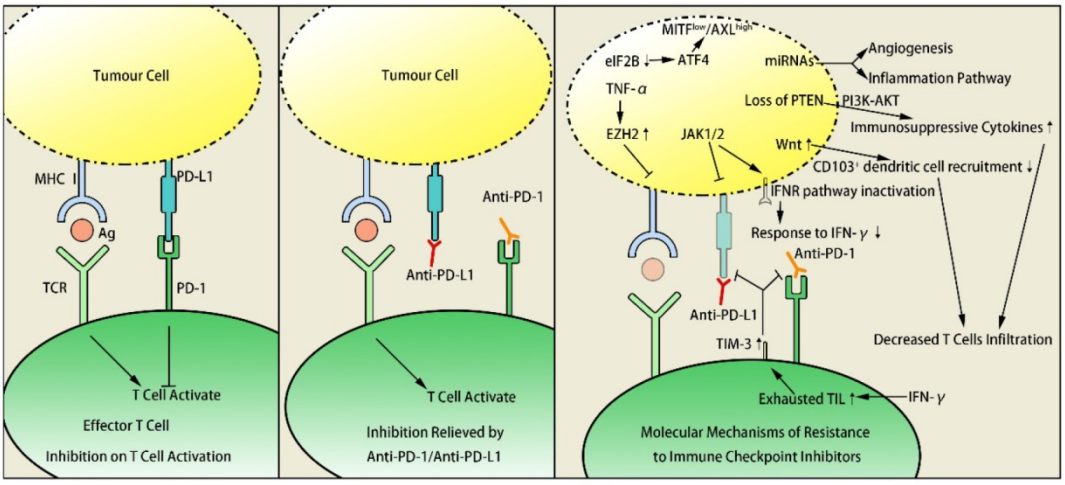

Figure 1. Molecular mechanisms of tumour resistance to immune checkpoint therapy

MITF suppression

MITF is an oncogene of melanoma, which negatively correlates with tumor invasion ability. Low expression of MITF increases melanoma invasion, making tumor cell possess characteristics of tumor stem cells[27], thus promotes the transformation of melanoma into a more invasive phenotype. This transformation contributes to tumor metastasis and immunotherapy resistance. Inhibition of translation initiation factor eIF2B leads ATF4 to activate AXL and suppress MITF, which increases MITFlow / AXLhigh drug-resistant phenotype, causing resistance in adoptive T-cell and anti-PD-1 immunotherapy[28].

Ezh2 upregulation

Histone methyltransferase Ezh2 controls resistance to T cell immunotherapy as a molecular switch in melanoma[29]. T cell infiltration was positively associated with Ezh2-PRC2 complex activity in human skin melanoma cells according to research summary of Cancer Genome Atlas database. After treatment of anti-CTLA-4 mAbs or IL-2 in mice, intratumoral TNF-α and T cells accumulation promoted the Ezh2 expression, which resulted in tumor immunogenicity loss, antigen expression silence and immunotherapy resistance. It was concluded from this experiment that Ezh2 inactivation reversed the resistance, increased curative effect of anti-CTLA-4 mAbs or IL-2 and inhibited melanoma growth significantly.

microRNA deregulation

miRNAs control tumor cell signal conduction by regulation of target genes, functioning similar to oncogenes or anti-oncogenes. miRNAs not only influence tumorigenesis and development but also play a part in resistance to targeted or immune therapy[30]. Fattore L and his coworkers defined a group of miRNAs closely related to melanoma progressing and recognized main downstream pathways. miRNAs reveal acquired resistance to MAPKi as well as innate resistance to anti-PD-1 immunotherapy, which are both associated with alterations of angiogenesis and inflammatory pathways[31,32]. In lung cancer, miR-8 family is able to target the PD-L1, increasing the efficacy of $CD8^+$T cell and tumor immunosurveillance, meanwhile, the upregulation of the immune suppressive PD-L1/PD-1 axis could be caused by the downregulation of these miRNAs in melanoma[32]. Expression of PD-L1 in lung cancer cells and melanoma cells could be unregulated by miR-8 family related miRNAs, causing immunotherapy resistance[31].

Loss of PTEN

Research on melanoma in animal models confirmed that loss of PTEN decreased T cells infiltration and increased the expression of immunosuppressive cytokines at tumor sites. Loss of PTEN induces VEGF expression and subsequently leads to immune suppression, which is confirmed by clinical samples and B16 murine tumours that the blockade of VEGF results in increased trafficking of T cells[33]. PTEN loss also inhibited autophagy, which decreased effect of T cell mediated tumor-killing and anti-PD-1 therapy[34]. Furthermore, human studies approved the strong relation between PTEN loss and pembrolizumab resistance[35]. Loss of PTEN promoted immune resistance through phosphatidylinositol 3-kinase (PI3K) - protein kinase B (AKT) pathway activation, which could be alternated by PI3K inhibitors. Therefore, combination of anti-PD-1 therapy and PI3K inhibitors applies to immunotherapy resistance from a new perspective[34].

TIM-3 upregulation

Both PD-1 and TIM-3 are immunologic checkpoint receptors expressed on tumor infiltrating lymphocyte (TIL). TIM-3 is an inhibitory molecule which has close connection to immune tolerance and T cell exhaustion in tumours[36], at the same time, its ligand Galectin-9 is secreted by epithelial cells in the thymus and mediates T cell apoptosis[37]. TIM-3 was significantly upregulated in TIL in tumor mouse models resistant to anti-PD-1 therapy[38]. Clinical trials in adaptive resistant patients of anti-PD-1 therapy showed TIM-3 upregulation and disconnection of anti-PD-1 mAbs with T cells. TIM-3 was highly expressed in dysfunctional TIL in human HNSCC. Dampened AKT/S6 phosphorylation in $PD\text{-}1^+TIM\text{-}3^+$ TIL led to weaken curative effect of anti-PD-1 treatment[39]. Adaptive resistance to anti-PD-1 mAb was overcome after addition of anti-TIM-3 mAb. This suggested TIM-3 and PI3K/AKT as promising targets of tumor immunotherapy.

JAK1/2 mutations

JAK1/2 dysfunction induced by mutations leads to acquired resistance to anti-PD-1 therapy. In human melanoma cell lines lack of PD-L1 expression, JAK1/2 mutations were found in 2 out of 48 cell lines[40]. Patients with JAK1/2 loss-of-function alterations might present poor prognosis according to data of Cancer Genome Atlas database. In melanoma and dMMR colon cancer patients with anti-PD-1 resistance, high JAK1/2 mutation load were detected[40]. Zaretsky J.M also found similar function loss in metastatic melanoma patients, suggesting the association between acquired resistance to anti-PD-1

therapy and JAK1/2 dysfunction[41]. The dysfunction of JAK1/2 led to PD-L1 expression absent or significantly down-regulated by IFN-γ receptor pathway inactivation. Deficiency of immunotherapy target resulted in resistance to anti-PD-1/ PD-L1 mAbs. Combination of IFNγ$^+$ T helper type 1 inducing cancer vaccines with PD-1 immune checkpoint blockade led to increase of PD-L1 expression[42]. The synergetic effect of combined therapy overcame immunotherapy resistance to some extent.

Cbl-b deficiency

Cbl-b is an E3 ubiquitin ligase that regulates T cell activation negatively. PD-L1 Ig not only suppresses T cell proliferation but also inhibits secretion of IFN-γ. Study on mice showed that Cbl-b WT mice were sensitive to PD-L1 Ig while Cbl-b$^{-/-}$ mice were not. In coculture of Cbl-b$^{-/-}$ CD8$^+$T cells and WT CD8$^+$ T cells, the existence of Cbl-b$^{-/-}$ CD8$^+$T cells diminished PD-L1 Ig mediated suppression of WT CD8$^+$ T cells. WT mice had increased sensitivity to treatment and fewer liver metastases while Cbl-b$^{-/-}$ mice showed no curative effect. The experimental results suggested that the Cbl-b$^{-/-}$ phenotype is one of the key factors of anti-PD-L1/PD-1 resistance or tumor progression[43].

B2M truncating mutation

B2M is associated with the heavy chain of major histocompatibility complex (MHC) I and the mutations of B2M may impact MHC I antigen presentation. Point mutations, deletions or loss of heterozygosity were detected in 29.4% in 17 progressing metastatic melanoma patients treated with ICBs. Also, the study suggested that B2M loss of heterozygosity enriched threefold in non-responders (30%) compared to responders (10%) along with poorer overall survival in independent cohorts of melanoma patients treated with anti-CTLA4 and anti-PD1 respectively. It is concluded that the occurrence of B2M truncating mutation results in resistance to immunotherapy[26].

Wnt/β-catenin signaling pathway activation

The activity changing of Wnt signaling is involved in multiple human diseases, including cancer, bone defects, schizophrenia, and arthritis[44]. Wnt1gene, a secreted cysteine-rich protein with the potential to act as a signaling molecule, is confirmed to induce breast tumor in mouse models[45]. Spranger revealed the correlation between activation of WNT/ β-catenin signaling pathway and absence of T-cell gene expression signature in human metastatic melanoma samples[46]. By mediating immune exclusion in melanoma caused by defective recruitment of CD1031 dendritic cells, the activation of Wnt /β-catenin signaling pathway resulted in resistance of immune-based therapies including anti-CTLA-4 and anti-PD-L1 mAbs[46].

Other mechanisms

Study on metastatic melanoma patient collected samples from 13 anti-PD-1 resistant patients and 3 patients resistant to combination of anti-PD-1 and ipilimumab therapy. In most resistant patients, density of VISTA$^+$ (12/18), PD-L1 expression (11/18) and intratumoral expression of FOXP3$^+$ were detected. In a small part of patients, PTEN loss (5/18), downregulation of HLA-A (4/18) and HLA-DPB1 (3/18) were found, which suggested the close relations between above indexes and acquired resistance to anti-PD-1 treatment[47]. Vivo imaging could reveal the real-time activity of anti-PD-1 mAbs at subcellular resolution in mice. Tumor-associated macrophages engulfed anti-PD-1 mAbs swiftly and the phagocytosis depended both on mAbs' Fc domain glycan and Fcγ receptors expressed by host myeloid cells. Before applying anti-PD-1 mAbs, blockade of Fcγ receptors prolonged the combining of mAbs and tumor-infiltrating CD8$^+$ T cells and enhanced tumor regression[48]. Loss of IFN-γ genes were found in ipilimumab resistant patients while knockdown of IFN-γ receptor 1 could impair the curative effect of anti-CTLA-4 therapy, indicating defect of IFN-γ pathways as a mechanism of anti-CTLA-4 therapy resistance[49].

Countermeasures of immunotherapy resistance

There are many strategies to overcome the resistance to immunotherapy, such as combination of chemotherapy, radiotherapy and tumor vaccine, etc. Agonists and inhibitors of specific receptors and molecules can also be applied to optimize immune therapy and overcome resistance.

PRRs agonist

Pattern recognition receptors (PRRs), a kind of pathogen associated molecular patterns recognition molecule on surface of innate immune cells, have effects of regulatory, phagocytosis, complement activation, inflammatory signal transduction, and inducing apoptosis. PRRs act as costimulatory molecules of macrophages and dendritic cells. Natural endo/ exogenous or synthetic PRRs agonists activate phagocytosis and antigen presentation of macrophages and myeloid cells in the tumor microenvironment. Preclinical models proved that PRRs agonists could overcome the resistance to anti-CTLA-4, anti-PD-1 and anti-PD-L1 targeting T-cell immune checkpoints[50].

ARNAX

ARNAX, a tumor vaccine, can induce anti-tumor CTLs without systemic cytokine/interferon production by increasing the efficacy of anti-PD-L1 as Toll-like receptor 3 agonist. It can also improve immunity of T helper type 1 in tumor microenvironment and enhance recruitment of dendritic cells, T cells and natural killer cells. Combination of ARNAX and anti-PD-L1 activates tumor-specific CTL then enhances CTL infiltration, which would overcome resistance to anti-PD-L1. Human research showed ARNAX$^+$ antigen induced and increased proliferation of antigen-specific CTL in peripheral blood mononuclear cells, indicating the potential of ARNAX to synergize anti-PD-1/PD-L1[51].

CpG oligonucleotide

CpG oligonucleotide function as a stimulator in the presence of anti-PD-1, inducing IFN, T-cell-tropic chemokines, and DC maturation thereby generating systemic T cell response to infiltrate tumor[52]. Anti-PD-1 treatment induces CpG-mediated tumor-specific CD8$^+$ T cells to differentiate into CD127^{high}KLRG1^{low} long-lived memory precursors preferentially. Intratumoral CpG oligonucleotide administration improves the quantity and quality of tumor-specific CD8$^+$ T cells, increasing sensitivity of anti-PD-1[52].

Oncolytic peptide LTX-315

The oncolytic peptide LTX-315 could promote the release of danger signals (DAMPs) such as ATP, Cytochrome C and HMGB1 and then cause plasma membrane disruption, mitochondria perturbation as well as cell death, acting as an immunogenic cytotoxic compound[53]. LTX-315 improves antitumor immunity by decreasing local immune suppressive T regulatory cells and myeloid-derived suppressor cells, leading to increase in CTLA-4 and decrease in PD-1 expression. The study of Yamazaki proved that intratumoral rejection of LTX-315 caused tumor regression or disappearance, offering the theoretical support to the combination of ipilimumab and LTX-315[54].

IDO inhibitors

Indoleamine 2,3-dioxygenase (IDO) is the rate-limiting enzyme of tryptophan catabolism, which is highly expressed in immune tolerant tumor tissues. In melanoma cells in IDO knockout mice treated with anti-CTLA-4 mAbs, tumor proliferation was inhibited and overall survival increased compared with wild-type mice, which was also observed in anti-PD-1/PD-L1 and glucocorticoid-induced tumor necrosis factor receptor therapy[55]. Combination of anti-CTLA-4 mAbs and IDO inhibitors reduced the inhibition of IDO on T cells and enhanced tumor-specific effector T cells infiltration. Hence, combination of IDO inhibitors is able to increase therapeutic effect of ICBs[55]. The synergistic effect has been approved by multiple clinical trials[56,57,58].

Combination of radiotherapy

During anti-PD-1 treatment in Kras-mutated p53-deficient lung cancer murine, PD-L1 expression on resistant and sensitive tumor cell showed no obvious difference. MHC I/II molecules on resistant tumor cells was downregulated significantly, along with TIL and IFN-γ decrease in microenvironment compared to sensitive tumor cells. However, local radiotherapy promoted IFN-β secretion and upregulated expression of MHC I molecules on the surface of resistant cells. Results proved that adjuvant radiotherapy helps to get over anti-PD-1 resistance, and then enhances efficacy of anti PD-1 immunotherapy[59].

anti-TIM3 Ab TSR-022

Upregulation of second immune checkpoint TIM-3 has been discovered in anti-PD-1 Ab-resistant tumors[39]. The Phase I clinical trial of anti-TIM3 Ab TSR-022 is ongoing (ID# NCT02817633), as a monotherapy and in combination with an anti-PD-1 antibody, in patients with advanced solid tumors who have limited available treatment options. Further experiments are required for anti-TIM3 Ab to clarify its unique value.

anti-LAG-3 Ab

Lymphocyte activation gene 3 (LAG-3) is a gene functioning as an inhibitory receptor on T cells, which increases the effect of regulatory T cells and shows relationship with T cell exhaustion[60]. Combining anti-LAG-3 Ab and anti-PD-1 Ab synergistically enhances T cell activity, and phase I/II clinical trial about the combination treatment is ongoing (ID#NCT01968109).

Other countermeasures

In addition to above strategies, gene detection[21,22], blocking PI3K-AKT, blockade of Fcγ receptors[47] and modulation of the gut microbiome[61] are also feasible. In patients with PD-1 resistant melanoma, upregulation of a few immune related genes results in immunosuppression[32], angiogenesis[34], monocyte and macrophage chemotaxis[49], extracellular matrix remodeling[61] and epithelial mesenchymal transition[61,62]. Therefore, combined blockade of these genes and PD-1 may administer to overcome anti-PD-1 resistance, providing better effects of antitumor immunotherapy[63] (Table 1) (Figure 2).

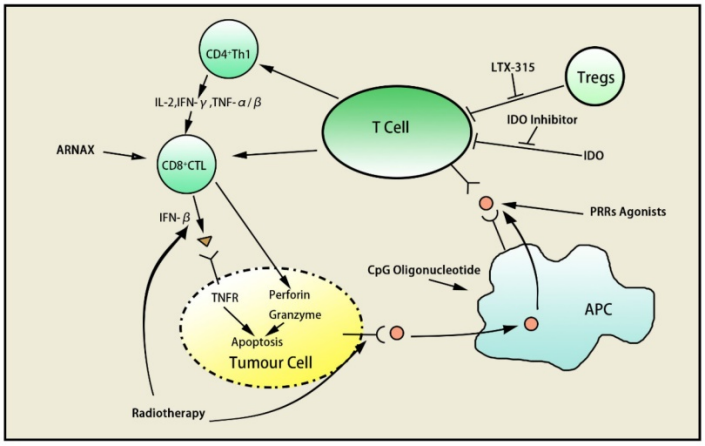

Figure 2. Countermeasures of immunotherapy resistance

Table 1. Strategies to overcome resistance to immune checkpoints blockade in cancer

mechanism	target blocking	T cell synergism	antigen presentation majorization	others
strategy	TSR-022	ARNAX	PRRs agonist	gut microbiome modulation
	blocking PI3K-AKT	CpG oligonucleotide	radiotherapy	LTX-315
	blocking FcγR	IDO inhibitors		
	gene detection	LTX-315		

Conclusion and perspectives

Immunotherapy is a new choice for cancer patients and has an unparalleled advantage compared to traditional treatment. Overcoming resistance to immunotherapy helps patients to get more benefit from this novel treatment thereby improving survival and life quality ultimately. Choosing valid biomarkers, whole-exome sequencing, analysis of gene expression, and seeing the whole forest of immunotherapy contributes to responders identification, resistance overcoming and resistant patients cure, which worth further exploration in the future.

Abbreviations

ICBs: immune checkpoint blockers; mAb: monoclonal antibody; CAR-T: chimeric antigen receptor T-cell immunotherapy; NSCLC: non-small cell lung cancer; HNSCC: head and neck squamous cell carcinoma; MITF: microphthalmia-associated transcription factor; Ezh2: enhancer of zeste homolog 2; TIM-3: T cell immunoglobulin mucin-3; Cbl-b: Casitas B-lineage lymphoma-b; B2M: β-2-microglobulin; PI3K: phosphatidylinositol 3-kinase; AKT: protein kinase B; TIL: tumor infiltrating lymphocyte; PRRs: pattern recognition receptors; IDO: indoleamine 2,3-dioxygenase.

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Author Contributions

Qin Li contributed to the design of the review and revised the article, had full access to all of the contents included in this study and took responsibility for the integrity of the data and the accuracy of the data analysis. Li Li provided the guide of immune knowledge, Xiaoyue Jiang wrote the main manuscript text and supplied the figures. All authors agreed to be accountable for the content of this paper.

Competing Interests

The authors have declared that no competing interest exists.

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