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T cell receptor signaling and cell immunotherapy

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Abstract: As the major sensor of adaptive immune system, T cell receptor (TCR) can recognize diverse antigens and initiate specific immune responses against invading pathogens and cancer cells. Understanding the mechanisms of TCR signaling has led to the development of engineered T cell therapies, which have extensive applications in the treatment of diseases such as cancer, infection and autoimmunity. Here, we review the current understanding of antigen-induced TCR signaling and discuss its applications in T cell therapies.

Keywords: T cell receptor-CD3 complex, signaling, T cell therapy

INTRODUCTION

As an indispensable part of the adaptive immune system, T cells are responsible for recognizing non-self antigens carried by pathogens and cancer cells to mount immunosurveillance [1]. T cell receptor (TCR) is the primary antigen sensor on the surface of T cells and its signaling is essential for T cell development and functions. Based on the expression of TCR types, T cells can be categorized into two major types: $\alpha\beta$ T cells and $\gamma\delta$ T cells, with $\alpha\beta$ T cells constituting more than 95% of the T cell population [2,3]. Therefore, $\alpha\beta$ T cells have been the research focus in recent decades, and here we mainly discuss the works of $\alpha\beta$ TCR (TCR, hereafter).

ORGANIZATION AND STRUCTURE OF TCR

In order to effectively navigate the intricate external and internal environment, TCR needs to recognize extremely diverse antigens presented by major histocompatibility complexes (MHCs) with specificity and sensitivity [4–8]. Therefore, TCR has evolved into one of the most sophisticated cell surface receptors composed of four dimeric subunits, including the antigen-recognizing subunit TCR $\alpha\beta$, and signaling subunits CD3 $\gamma\epsilon$, CD3 $\delta\epsilon$, and CD3 $\zeta\zeta$ (Figure 1).

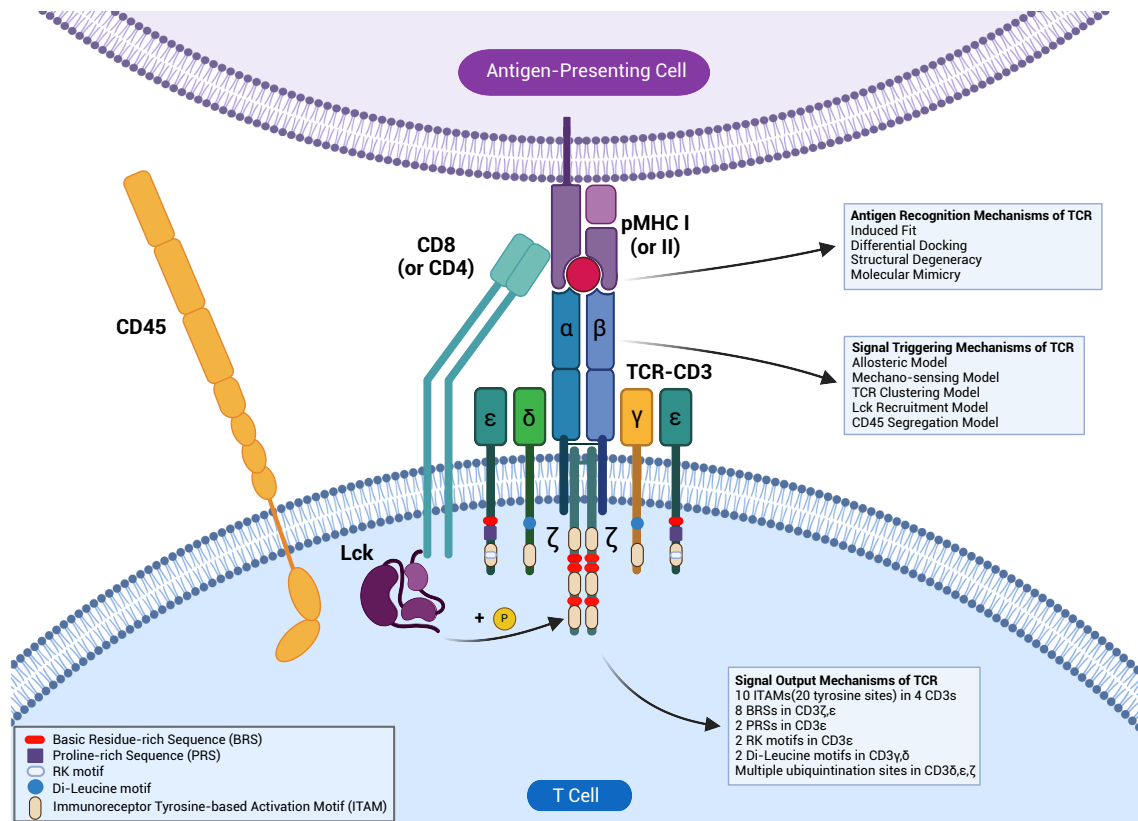


Figure 1 Structure, antigen recognition, triggering and signal output mechanisms of TCR-CD3 complex.

The antigen-recognizing subunit $TCR\alpha\beta$ is composed of an α chain and a β chain connected by disulfide bonds. Each chain contains an extracellular variable region (V region) domain, a constant region (C region) domain, a transmembrane domain, and a short intracellular domain [3,9]. The C region domain and the transmembrane domain are responsible for the assembly of the TCR complex and the subsequent transmission of antigen signals in the TCR complex, while the short intracellular domain is generally considered to be non-functional. The V region domain is the functional region involved in antigen recognition, containing three complementarity-determining regions (CDR): CDR1, CDR2, and CDR3 [10]. Producing variable regions (V regions), diversity regions (D regions), and joining regions (J regions), the $TCR\alpha$ gene includes $V\alpha$ and $J\alpha$ segments, and the $TCR\beta$ gene includes $V\beta$, $D\beta$, and $J\beta$ segments. V(D)Js are linked by non-homologous end joining with the assistance of RAG recombinase and DNA repair proteins, resulting in diverse $TCR\alpha$ and $TCR\beta$. The paired heterodimers of α and β chains further increase the variability of combinations [10–12]. Theoretically, V(D)J recombination can generate more than 10^{15} possible TCRs in human species, and an individual has approximately 2×10^7 TCRs after thymic selection [11,13–15]. CDR1 and CDR2 are encoded by the V region of TCR, and mainly interact with the terminus of the antigen peptide and the α -helix on the surface of MHC, respectively [15–17]. CDR3, the most variable among the three CDR regions, is encoded by the V(D)J junction and is responsible for recognizing various antigen peptide.

Due to the short cytoplasmic domain of $TCR\alpha\beta$, the function of transmitting antigenic signals is exerted by $CD3\gamma\epsilon$, $CD3\delta\epsilon$, and $CD3\zeta\zeta$ [18,19]. The extracellular regions of $CD3\gamma$, $CD3\delta$, and $CD3\epsilon$ consist of Im-

munoglobulin (Ig)-like domains, whereas the extracellular region of CD3 ζ is very short. The α chain and the β chain interact with CD3 chains through electrostatic and hydrophobic interaction in their transmembrane domains. These domains collectively mediate the assembly of the TCR complex and potentially regulate the transmission of subsequent antigenic signals within the complex. The intracellular regions of the CD3 chains all contain immunoreceptor tyrosine-based activation motifs (ITAMs), and each ITAM contains two tyrosine sites that can be phosphorylated to facilitate signal transduction into the cell. CD3 γ , CD3 δ , and CD3 ϵ each have one ITAM, while CD3 ζ contains three ITAMs [20]. Additionally, apart from ITAMs, CD3 γ and CD3 δ each have a Di-Leucine motif, CD3 ϵ has a basic residue-rich sequence (BRS), an “RKGQRxxY” (RK) motif, and a proline-rich sequence (PRS), and CD3 ζ contains multiple BRSs between ITAMs. All of these motifs may play a role in signal transduction [21,22] (Figure 1).

In 2019, the cryo-electron microscopy (cryo-EM) structure of the TCR-CD3 complex with a resolution of 3.7 Å was reported, revealing a detailed structural assembly mechanism of TCR $\alpha\beta$ /CD3 $\delta\gamma$ /CD3 $\gamma\epsilon$ /CD3 $\zeta\zeta$ with the stoichiometric ratio of 1:1:1:1 [23]. This structure revealed the folding of each protein, the formation of intramolecular and intermolecular disulfide bonds, the interaction between the extracellular Ig-like domains of each protein, and the involvement of salt bridges and hydrophobic interactions in the transmembrane regions. Additional cryo-EM structural analysis unveiled a cholesterol-binding tunnel (CBT) formed by TCR α/β and CD3 ζ/γ , capable of recognizing two cholesterol molecules. This suggests that cholesterol is involved in the assembly of the transmembrane region [24]. A recent work on the cryo-EM structure of TCR-CD3 complex in a native-like nanodisc lipid bilayer revealed new “closed and compacted” conformations that are distinct from previously reported structures of receptors in detergent. The conformations showed a “closure” of the TCR extracellular domains against the CD3 extracellular domains and a “compaction” of the juxtamembrane linker peptides that contact with the outer leaflet of the membrane, accompanied by rotations of TM helices. These conformations may represent the unliganded resting state for the TCR-CD3 complex *in vivo*, and suggest a membrane environment-mediated allosteric mechanism upon antigen triggering [25]. Nonetheless, the information regarding the critical intracellular regions of the signaling CD3s is lacking due to their dynamic nature. It should be pointed out that the intracellular domain of CD3 ϵ and CD3 ζ carrying BRSs can interact electrostatically with acidic phospholipids in the inner-leaflet layer of the plasma membrane, and this interaction induces amphipathic helical folding of ITAMs [26–30]. Consequently, critical tyrosine residues become embedded in the hydrophobic core of the membrane bilayer [27,29]. These protein-lipid interactions lock the TCR in a resting or inactive conformation, while also facilitating the formation of TCR oligomers to rapidly transmit antigenic signals [31–35].

DIVERSE ANTIGEN RECOGNITION MECHANISMS OF TCR

The antigen recognition process of TCR involves TCR-antigen and TCR-MHC interactions. Due to the tremendous variations of each module, there is a high level of diversity at the interface between TCR and peptide-MHC complex (pMHC). Several antigen recognition mechanisms have been proposed and summarized, including induced fit based on TCR and pMHC plasticity or flexibility, differential docking, structural degeneracy, and molecular mimicry [36–39] (Figure 1).

In general, TCR can interact with different pMHC by adopting different docking angles and changing

conformations bilaterally to accommodate TCR-pMHC interaction. Various factors, such as shape complementarity, hydrogen bonding, salt bridging and Van der Waals force, can redundantly create similar physical and chemical characteristics that allow for the tolerance of sequence substitutions in paired antigens and TCRs. This sequence tolerance, named as cross-reactivity, enables a limited number of TCRs to recognize a larger pool of antigens but also increases the risk of undesired recognition [36]. For example, exposure to a pathogen can alter the immune response to a subsequent unrelated pathogen, resulting in either protective or pathogenic outcomes [40].

DIVERSE TCR TRIGGERING MECHANISMS

The TCR triggering process is to convert the antigen signal from the extracellular antigen-recognizing domain of TCR $\alpha\beta$ to the intracellular domains of CD3 in order to recruit downstream proteins. Previous studies have primarily focused on exploring the mechanism by which antigen signals are converted into phosphorylation signals, as the recruitment of downstream proteins by CD3 mainly relies on the phosphorylation of ITAMs. Lck and Fyn kinases mediate the phosphorylation process of the CD3 chains, while CD45, SHP-1, and SHP-2 phosphatases mediate the dephosphorylation process [41–44]. In essence, TCR triggering involves modulating the phosphorylation/dephosphorylation equilibrium by either promoting kinase-mediated phosphorylation or inhibiting phosphatase-mediated dephosphorylation.

There are five major models of TCR triggering, addressing the question from either physical or chemical angle. The physical models include the Allosteric and Mechano-sensing model, while the chemical models include TCR clustering, Lck recruitment, and CD45 segregation (Figure 1). It is worth noting that these models are not mutually exclusive but can act synergistically to enhance sensitivity and specificity.

Allosteric model

The interaction between pMHC and TCR complex leads to conformational changes in TCR extracellular, transmembrane and intracellular domains of TCR. These changes occur in not only the antigen-recognizing region but also the TCR α AB loop, the TCR β FG loop, the cholesterol-binding site in the transmembrane region, the TCR α transmembrane region, the transmembrane region of CD3 $\zeta\zeta$, and the intracellular region of CD3 ϵ [45–50]. These changes facilitate the phosphorylation process by allowing easier access for Lck to the CD3 ITAMs. Recently, the structure of TCR complexed with monomeric pMHC, lacking the intracellular CD3 regions, showed no obvious difference compared to mock TCR, except the CDRs [51,52]. However, recent investigation revealed closed and compacted conformations of TCR-CD3 complex in a more physiological lipid membrane, which may represent the true resting state, hypothesizing that structures of TCR-CD3 complex in detergent are approximately ligand-activated conformation even without ligand [25]. Further investigation is needed to confirm or refute this proposed model.

Mechano-sensing model

When recognizing pMHC, TCR also senses the mechanical forces, including the possibilities of pull force,

shear force and tension force [53–56]. These forces aid in the formation of catch or slip bonds between TCR and pMHC, facilitating antigen discrimination by potentially disrupting CD3-lipid interactions to release ITAMs for phosphorylation [53–55].

TCR clustering model

Like other receptors, TCR has been shown to form dimers or oligomers after stimulation and subsequently generate an immunological synapse to promote triggering [57,58]. Although a recent study using single-molecule imaging argues that monomeric TCR drives antigen recognition and TCR clusters have enrichment of TCR monomers rather than oligomers, it is possible that TCR molecules form spatial clusters but remain structurally monomeric [59]. Indeed, a series of DNA origami-based assays showed that the spatial control of antigen or receptor clustering regulates TCR and chimeric antigen receptor (CAR) activation, as the formation of TCR clusters enables serial engagement of multiple receptors by the same ligand, thus increasing the avidity of antigen stimulation [60–64]. Overall, TCR clustering may not directly affect initial triggering, but could amplify the initial signal to enhance antigen sensitivity.

Lck recruitment model

In addition to TCR, pMHC also interacts with Lck-bound CD4/8 coreceptors to increase the local concentration of substrates and kinases [57]. pMHC binds to TCR at a tilted angle, and the coreceptors can only fit underneath of the pMHC complex in one direction to bring Lck into proximity [65]. TCRs with reverse docking topology on pMHC cannot trigger signaling even with high binding affinities [65]. Apart from coreceptors, Lck can also be recruited to the TCR through CD3 ϵ . The BRS and RK motifs in CD3 ϵ , which are bound to the membrane at the resting stage, can be released upon antigen engagement and recruit Lck through ionic interactions [30,66]. In mature T cells, free Lck is shown to be responsible for initial TCR triggering, while coreceptor-bound Lck is recruited later, possibly due to the higher mobility of free Lck [67,68]. Preventing coreceptor involvement reduces antigen sensitivity but does not completely abolish TCR signaling [69].

CD45 segregation model

The interaction between pMHC and TCR results in a close contact area between T cells and antigen-presenting cells. This tight contact prevents the CD45 phosphatase, which has a rigid and extended extracellular domain, from entering the interface due to steric hindrance. As a result, the dephosphorylation process is impaired [42,70]. As the primary TCR phosphatase, CD45 has multiple splicing variants expressed at different T cell stages. Even the shortest CD45 variant CD45RO has a dimension of approximately 21 nm, larger than that of the TCR-pMHC complex at approximately 14 nm. Indeed, CD45 segregation has been observed in TCR-enriched microvilli and lately formed immunological synapses [71,72]. Recent studies have shown that promoting CD45 segregation by controlling ligand positioning, height or slowing TCR diffusion can trigger T cells [73–75]. Interestingly, CD45 can be pre-excluded from TCR-enriched microvilli before antigen engagement, and this exclusion is dependent on the relatively short transmembrane region

rather than the CD45 extracellular domain, indicating that size exclusion acts together with other mechanisms to determine protein partitioning [76]. However, the CD45 segregation model cannot fully explain the non-stimulatory feature of reverse docking antigen discussed above, suggesting that both Lck recruitment and CD45 segregation are necessary to trigger TCR phosphorylation.

DIVERSE SIGNAL OUTPUTS OF TCR

The TCR signal output is dependent on the intracellular domains of the CD3 chains, which exhibit functional diversity (Figure 1). CD3 γ and CD3 δ each mainly contain an ITAM and a di-Leucine motif. CD3 ϵ contains a BRS, PRS, RK and ITAM motif, while CD3 ζ contains three BRSs and three ITAMs.

As previously mentioned, the BRS motif mediates the interaction of the intracellular domain of CD3 with the inner leaflet of the plasma membrane, thereby keeping the ITAMs in an autoinhibitory state. Interestingly, when membrane binding is disrupted, the exposed CD3 ϵ motifs, including the BRS, RK and PRS motifs, can synergistically interact with Lck to promote CD3 phosphorylation [30,66]. CD3 ζ BRS can also interact with Lck with weaker affinity [30]. Additionally, CD3 ϵ BRS can interact with the p85 subunit of PI3K to regulate the Akt signaling pathway, and CD3 ϵ PRS can interact with Nck to regulate immune synapse maturation and T cell activation [49,77]. Differently, the di-Leucine motifs regulate the downmodulation or internalization of surface TCRs, and CD3 ubiquitination also regulates cycling and degradation [21,78–86]. The ubiquitination sites have been reported to be located in CD3 δ , CD3 ϵ , and CD3 ζ , which regulate both the activation and the development of T lymphocytes [81–86].

There are ten ITAMs and six types of ITAMs in one TCR molecule, which could generate diverse phosphorylation combinations theoretically. It is known that strong and weak antigens elicit distinct T-cell responses, including cytokine production, proliferation and differentiation [87,88]. Mechanistically, strong antigens cause full phosphorylation of CD3 ζ ITAMs, while weak antigens can only trigger partial phosphorylation at the same concentration [89]. Mouse models containing various ITAM mutations (from tyrosine to phenylalanine) have been constructed to study the quantitative and qualitative signaling of CD3 ITAMs. Cytokine production of T cells appears to require only a small number of phosphorylated ITAMs, whereas cell proliferation requires more [90,91]. It is worth mentioning that CAR-T cells carrying only CD3 ζ ITAM1 are more likely to differentiate into the memory phenotype, while CAR-T cells carrying only CD3 ζ ITAM3 maintain a more naive phenotype [92]. Moreover, compared with conventional CD3 ζ CAR, CD3 ϵ , γ or δ CAR can improve anti-tumor performance through various mechanisms involved in activation potential, metabolism and stimulation-induced T cell dysfunctionality [93]. These findings all demonstrate that the diverse CD3 ITAMs can provide both qualitative and quantitative signaling to determine T cell functions [22,94–96].

The phosphorylation levels of the two tyrosines within an ITAM are not necessarily synchronized, leading to dual- and mono-phosphorylated states of an ITAM. Recent results from absolute quantitative mass spectrometry reveal that in CD3 ϵ ITAM, the phosphorylation level of the N-terminal tyrosine site is much higher than that of the C-terminal tyrosine. Unlike dual-phosphorylated CD3 ITAM, which recruits Zap70, mono-phosphorylated CD3 ϵ ITAM recruits Csk to limit Lck activity and TCR phosphorylation [77]. Similarly, mono-phosphorylated CD3 δ were found to recruit SHP-1 to restrain activation signals, thus pre-

venting T cell exhaustion and dysfunction [93].

It is widely accepted that various extrinsic and intrinsic environmental factors, including cytokines, costimulatory molecules, antigen-presenting cells and antigen dosage, are closely related to the phosphorylation of ITAMs within the TCR complex. However, the specific mechanism by which phosphorylated ITAM diversity is generated and affects downstream-pathway selection remains largely unclear.

TCR AND CAR-T IMMUNOTHERAPY

Based on the knowledge of TCR signaling, scientists have developed strategies of CAR-T cell therapy that have achieved significant successes in treating hematological malignancies and are being expanded to treat solid tumors, autoimmune diseases, viral infections, and fibrosis [97–101]. To improve the clinical performance of CAR-T cells, multiple generations of CAR have been developed, and more are under development [102–108].

The CAR is a chimera of an antibody-derived extracellular domain for target recognition, a transmembrane domain and an intracellular signaling domain. The transmembrane domain, which usually mediates dimerization, plays a crucial role in CAR-T cell activation. In the first generation CAR, CD3 ζ is directly linked to an extracellular antigen-recognizing domain and this construct tends to form homodimer or heterodimer with native TCR [104,109]. Later, the dimeric transmembrane domains of CD28 or CD8 were more widely used due to their higher expression stability than CD3 ζ transmembrane domain [110,111]. Recently, *de novo* designed transmembrane domains proved that different oligomeric states, from monomers up to tetramers, are positively correlated with the anti-tumor capacity of CAR-T cells, indicating a novel strategy to improve CAR-T cell therapy [112]. CAR_{Cys}-T cells using a 4-1BB-derived hinge region containing 11 cysteines residues form a larger diameter of CAR clusters upon antigen stimulation and showed promoted antitumor activity, which highlights the importance of CAR clustering [113].

Lck recruitment is a crucial step of TCR activation, as well as CAR activation. The tyrosine kinase inhibitor Dasatinib can make CAR-T cells function-off by inhibiting Lck activity [114]. The CD28 intracellular domain in the second-generation CAR can directly recruit Lck besides initiating co-stimulatory signal [115–117]. In addition, the transmembrane region of CD28 mediates CAR heterodimerization with native CD28 to recruit Lck and this mechanism may also apply for the CAR carrying CD8 transmembrane region [112,117,118]. Interestingly, transmembrane domain of inducible costimulator (ICOS) was newly found to recruit Lck, and thus may be applied for engineering CARs with enhanced functions [119].

Besides Lck recruitment, CD45 segregation is also important. CAR-T activation can be precisely tuned by manipulating the size of the extracellular domain of CAR or CD45, suggesting a novel strategy to regulate CAR-T immune response [120].

Overall, only lessons from the chemical model of TCR triggering can be learned, due to the structural difference between CAR and TCR. In addition to the TCR triggering model, the diverse signal output mechanism of TCR can also inspire designing more effective CARs. In the crucial CD3 ζ module, the number and position of ITAMs have been found to direct CAR-T cells to different effector or memory programs and yield distinct therapeutic profiles [92]. Besides engineering ITAM, mutating all lysine residues to inhibit ubiquitination markedly represses CAR downmodulation while enhancing the recycling of internalized

CARs. Such recycling CAR shows more robust proliferation, better killing capacity and memory T cell differentiation, leading to superior persistence *in vivo* [121].

The diversity of the four CD3 chains ensures subtle regulation of TCR signaling, which is not present in CAR signaling. Although CD3 ζ module is being used in most CAR designs, high throughput screening approaches have also revealed that CD3 ϵ can enhance the anti-tumor capacity of CAR-T cells [122], and that FCRL6-CD3 γ would increase killing rates of CAR-T cells [123]. A recent study showed that replacing the cytoplasmic domain of CD3 ζ with that of other CD3 chains, especially CD3 δ , would improve killing efficacy with lower expression of exhaustion markers and higher expression of memory and self-renewing markers [93]. Further, introducing the cytoplasmic domain of CD3 ϵ into CD28 and CD3 ζ based CAR reduced cytokine storm while improving CAR-T cell persistence and antitumor function. Similarly, in the 4-1BB and CD3 ζ based CAR context, incorporation of CD3 ϵ promoted CAR T cell function through Lck recruitment by the RK motif of CD3 ϵ [66,77]. Interestingly, another study found that 4-1BB-based CAR containing the PRS and ITAM motif of CD3 ϵ enhances *in vivo* antitumor function in settings of low antigen [124]. All these findings highlight that harnessing CD3 diversity can improve the performance of CAR-T cells.

To better mimic TCR signaling, the latest research has rebuilt CAR on native TCR-CD3 complex [125–131]. T cell antigen coupler (TAC) is composed of an antigen-recognizing domain, an anti-CD3 single chain antibody to co-opt native TCR, and a co-receptor domain; Antibody-TCR (AbTCR) combines a Fab-based antigen-recognizing domain with a $\gamma\delta$ TCR-signaling domain, avoiding the mispairing issues with traditional $\alpha\beta$ TCR; T cell receptor fusion constructs (TRuCs) offer an intact TCR complex by fusing antibody-based binding domain to TCR subunits; synthetic TCR and antigen receptor (STAR) or HLA-independent T cell receptors (HIT receptors) incorporate the antigen-recognizing domain of an antibody with the constant regions of TCR; TCR-like CAR (TCAR) designs involve arranging identical antigen-recognizing domain in tandem with the constant regions of TCR. These strategies have shown promise in inducing more efficient anti-tumor responses, such as higher antigen sensitivity, lower cytokine release, better proliferation, and less susceptibility to dysfunction. It is worth noting that these CARs abandon the co-stimulatory modules, and may therefore fit for different treatment scenarios. All the above studies have proved that the investigation of TCR activation mechanisms plays an essential role in guiding the design of CAR-T therapy.

TCR AND TCR-T IMMUNOTHERAPY

Different from CAR-T therapy, later developed TCR-T therapy has drawn more attention to the TCR antigen recognition mechanism, as the most significant challenge for TCR-T therapy is identifying individual-specific disease-associated TCR clones. Several algorithmic methods have been developed to group TCR clusters based on the sequence similarity of CDRs, particularly CDR3, and assist in predicting specific TCR clones. Some of these methods include grouping of lymphocyte interactions by paratope hotspots (GLIPH), TCRdist, antigen-specific lymphocyte identification by clustering of expanded sequences (ALICE), geometric isometry-based TCR alignment algorithm (GIANA), DeepTCR, pMHC-TCR binding prediction network (pMTnet) and others [132–141]. However, these methods do not incorporate the detailed structural mechanisms of antigen recognition mentioned above. With the breakthrough of cutting-edge deep neural network structure prediction methods like AlphaFold and RoseTTAFold, structure-based approaches

Table 1 Application of antigen recognition, triggering and signal output mechanisms in immunotherapies

Immuno-therapies	Applied mechanisms	Strategy of design	References
CAR-T	TCR clustering	Introducing dimeric CD3 ζ , CD28 and CD8 transmembrane domain.	[104,109–111]
	TCR clustering	Designing the transmembrane region to form multimerization (including trimerization and tetramerization).	[112]
	TCR clustering	Introducing a 4-1BB-derived hinge region containing 11 cysteines to form a larger diameter of CAR clusters.	[113]
	Lck recruitment	Introducing CD28 intracellular region to recruit Lck.	[115,116]
	Lck recruitment	Introducing CD28, CD8 and ICOS transmembrane domain to recruit Lck.	[105,112,118,119]
	CD45 segregation	Controlling the size of extracellular domain of CAR.	[120]
	Signal output mechanism	Engineering number and location of CD3 ζ ITAMs.	[92]
	Signal output mechanism	Mutating all lysine residues in the cytoplasmic domain.	[121]
	Signal output mechanism	Introducing CD3 ϵ , γ and δ cytoplasmic domain.	[66,77,93,122–124]
	Signal output mechanism	Building CAR on TCR to retain total CD3 diversity.	[125–131]
TCR-T	Antigen recognition mechanism	Grouping TCR clusters and predicting reactive TCR clones.	[132–143]
	Mechano-sensing mechanism	Engineering catch bonds between TCR and pMHC to improve sensitivity but reduce cross-reactions.	[145]
	Signal output mechanism	Co-transduction of ATAM to enhance proliferation and persistence.	[146,147]

represent a promising path for predicting TCR-pMHC interactions and identifying reactive TCR clones [142,143].

TCR-T therapy also references TCR triggering model. Due to the potential off-target toxicity of high-affinity TCRs in clinical trials, innovative therapeutic approaches are needed to maintain the effectiveness of TCRs in targeting tumors while minimizing their affinity for self-antigens [144]. The mechano-sensing model suggests that engineering the catch bond between TCR and pMHC can improve the sensitivity of TCR but reduce cross-reactions, making it a promising technology for TCR-T therapy [145].

The signal output mechanism is also applied to TCR-T therapy. TCR-T therapy has generally failed in clinical trials, due to defects such as limited expansion and short duration of transferred T cells. Co-transduction of artificial T cell adaptor molecule (ATAM) along with TCR into T cells has shown promise in enhancing the proliferation ability and persistence of TCR-T cells both *in vitro* and *in vivo* [146,147].

In general, CAR and TCR differ in terms of their structures, antigen recognition mechanisms, activation mechanisms, and clinical applications [22,148]. CAR-T therapies have demonstrated outstanding therapeutic effects on leukemia and lymphoma treatment, but have shown poor efficacy in solid tumor treatment. Although TCR-T therapy has shown better results in treating solid tumors in research studies, it also has challenges, such as TCR mismatch, non-specific cytotoxicity, and cytokine storm caused by immature technology [149]. Understanding the mechanism of TCR activation has inspired the development of strategies for more effective immunotherapies (Table 1).

SUMMARY AND OUTLOOK

TCR signaling provides the primary signal for T cells, directing the immune response against pathogens and

cancer cells. Systematic investigations of TCR activation mechanisms contribute to a thorough understanding of T-cell immunity. As mentioned above, TCRs have been found to employ diverse antigen recognition, triggering, and signal output mechanisms to effectively deal with various antigens (Figure 1). These mechanisms can be harnessed to develop more efficient TCR-T and CAR-T cell therapies against various diseases.

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Author contributions

C.X. and X.S. conceived and designed the topics. L.Z. and X.S. wrote the manuscript. X.X. and C.X. revised it.

Conflict of interest

The authors declare no conflict of interest.

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