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REVIEW

The mechanisms and clinical significance of CD8$^+$ T cell exhaustion in anti-tumor immunity

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ABSTRACT CD8$^+$ T cell exhaustion, a critical challenge in the immune response to cancer, is characterized by a profound decline in the functionality of effector CD8$^+$ T cells. This state of exhaustion is accompanied by the upregulation of various inhibitory receptors and significant shifts in both transcriptional and epigenetic profiles, thus ultimately leading to inadequate tumor control. Therapeutic strategies aimed at reversing CD8$^+$ T cell exhaustion have the potential to rejuvenate immune responses and enhance treatment efficacy. This review compiles current knowledge regarding the molecular mechanisms underlying CD8$^+$ T cell exhaustion, including the roles of immune checkpoint molecules, the tumor microenvironment, metabolic reprogramming, transcription factors, and epigenetic modifications. Emerging therapeutic approaches designed to combat CD8$^+$ T cell exhaustion are evaluated, with emphasis on the modulation of immune checkpoints; targeting of metabolic and transcriptional changes; and exploration of other innovative strategies, such as epigenetic editing and engineered CAR-T cells. Importantly, we expand the exhaustion concept to immune cells beyond CD8$^+$ T cells, such as CD4$^+$ T cells, natural killer cells, and myeloid populations, thereby highlighting the broader implications of systemic immunosuppression in the cancer context. Finally, we propose avenues for future research aimed at further elucidating the factors and molecular mechanisms associated with CD8$^+$ T cell exhaustion, thereby underscoring the critical need for strategies aimed at reversing this state to improve outcomes in cancer immunotherapy.

KEYWORDS CD8$^+$ T cell exhaustion; immune checkpoint; immune checkpoint inhibitors; cancer immunotherapy; anti-tumor immunity

Introduction

CD8$^+$ T cells mediate tumor control *via* major histocompatibility complex (MHC)-restricted antigen recognition and cytokine secretion. However, persistent antigen exposure in cancer leads to CD8$^+$ T cell exhaustion. Emerging evidence of striking heterogeneity within the exhausted CD8$^+$ T cell compartment has delineated 2 functionally distinct subsets: progenitor exhausted and terminally exhausted CD8$^+$ T cells. Progenitor exhausted CD8$^+$ T cells exhibit a stem-like phenotype; retain self-renewal ability; and respond to immune checkpoint blockade, thereby sustaining anti-tumor immunity. In contrast, terminally exhausted CD8$^+$ T cells upregulate multiple inhibitory receptors [such as cell death protein 1 (PD-1), T cell immunoglobulin (Ig) and mucin-domain containing-3 (TIM-3), T cell immunoreceptors with Ig and ITIM domains (TIGIT), lymphocyte activation gene 3 (LAG-3), and cytotoxic T-lymphocyte-associated protein 4, (CTLA-4)]; alter transcriptional and epigenetic reprogramming; demonstrate diminished proliferative potential and functional impairment (characterized by loss of cytotoxicity and cytokine production); and show resistance to current immunotherapies[1] (**Figure 1**). The causal relationship between tumor metabolic reprogramming and CD8$^+$ T cell exhaustion remains to be clarified.

CD8$^+$ T cell exhaustion occurs in various tumors. Exhausted T cells within the tumor microenvironment (TME) contribute to immune evasion and tumor progression, and frequently correlate with poor clinical outcomes[2]. Reversing this exhaustion is the cornerstone of modern immunotherapy based on immune checkpoint inhibitors (ICIs), such as anti-PD-1 and

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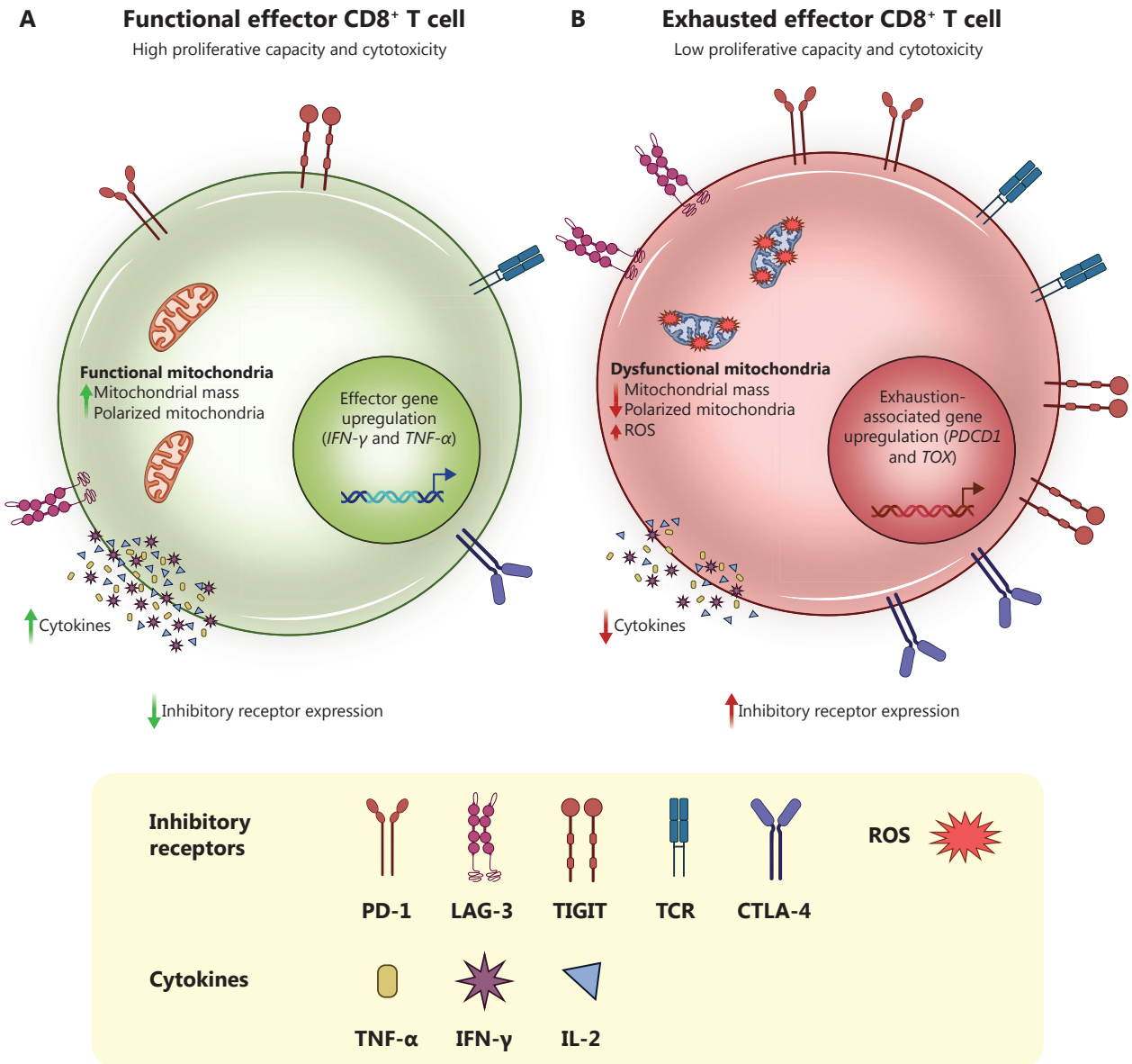


Figure 1 Functional and molecular hallmarks of CD8⁺ T cells. (A) Functional effector CD8⁺ T cells. Functional effector CD8⁺ T cells express *IFN-γ* and *TNF-α* genes under transcriptional and epigenetic regulation. The cells have low inhibitor receptor expression (PD-1, LAG-3, TIGIT, TCR, and CTLA-4) and produce pro-inflammatory cytokines (IFN-γ, TNF-α, and IL-2), which amplify immune responses. Meanwhile, they exhibit efficient functional mitochondrial metabolism through mitochondrial mass and polarized mitochondria. These elements together ensure CD8⁺ T cells' high proliferative ability and cytotoxicity for mounting immune responses. (B) Exhausted CD8⁺ T cells. Exhausted CD8⁺ T cells are characterized by pronounced expression of inhibitory receptors (PD-1, LAG-3, TIGIT, TCR, and CTLA-4) and diminished production of cytokines (IFN-γ, TNF-α, and IL-2). Exhausted CD8⁺ T cells exhibit mitochondrial dysfunction, accompanied by diminished mitochondrial mass and polarized mitochondria, and elevated ROS production. The cells show significantly elevated expression of exhaustion-associated genes (*PDCD1* and *TOX*) under transcriptional and epigenetic regulation. Tox is required for exhausted CD8⁺ T cells' epigenetic remodeling and survival. Exhausted CD8⁺ T cells display significantly diminished proliferative ability and cytotoxicity. CTLA-4, cytotoxic T-lymphocyte-associated protein 4; IL-2, interleukin-2; IFN-γ, interferon-gamma; LAG-3, lymphocyte activation gene 3; PD-1, programmed cell death protein 1; PDCD1, programmed cell death 1, also named PD-1; ROS, reactive oxygen species; TCR, T cell receptor; TIGIT, T cell immunoreceptors with Ig and ITIM domains; TNF-α, tumor necrosis factor alpha; TOX, thymocyte selection associated high mobility group box.

anti-CTLA-4 drugs designed to restore T cell function[3,4]. Emerging strategies such as next-generation CAR-T cell engineering and CRISPR-based epigenetic editing technologies have transformative potential to restore T cell functionality and overcome exhaustion, thus offering novel avenues for cancer immunotherapy. A critical unresolved question is whether this exhausted state reflects an adaptive differentiation intermediate or a terminal fate under chronic antigen exposure.

Whereas prior reviews have focused on single aspects of CD8+ T cell exhaustion, this review uniquely integrates multi-layered mechanisms into a unified framework. Herein, we discuss the multifaceted causes and molecular mechanisms underlying CD8+ T cell exhaustion. We outline the roles of immune checkpoint molecules, the TME, metabolic reprogramming, transcription factors, epigenetic modifications, and fibroblasts in CD8+ T cell exhaustion. Each element uniquely contributes to the exhaustion phenotype, and influences T cell activity and survival within the immunosuppressive tumor milieu. We further discuss potential therapeutic strategies to reverse CD8+ T cell exhaustion. In addition, we focus on the exhaustion of other immune cells, to highlight the systemic nature of cancer immunosuppression. Addressing these mechanistic and therapeutic questions should aid in developing more effective cancer immunotherapies.

Roles of immune checkpoint molecules in CD8+ T cell exhaustion

Key immune checkpoint molecules in CD8+ T cell exhaustion

Immune checkpoints critically govern CD8+ T cell functionality in anti-tumor immunity. Dysregulation of immune checkpoints often drives CD8+ T cell exhaustion *via* sustained overexpression of immune checkpoint molecules.

PD-1, which is expressed predominantly on the surfaces of exhausted CD8+ T cells, binds its ligand PD-L1, and subsequently inhibits TCR signaling and downstream effector function. This binding interaction impairs cytotoxic capabilities, cytokine production, and proliferation, and contributes to the anergic state of T cells[5]. CTLA-4 competes with the co-stimulatory molecule CD28 for binding to B7 ligands on antigen-presenting cells (APCs), and consequently attenuates TCR signaling, decreases IL-2 production, limits T cell activation, and promotes exhaustion[6].

LAG-3, another important immune checkpoint molecule, binds MHC class II molecules and delivers inhibitory signals that further dampen T cell responses[7]. The finding that coexpression of LAG-3 and PD-1 is associated with a more severe exhaustion phenotype highlights LAG-3's synergistic role in promoting T cell exhaustion[8]. TIM-3, which binds galectin-9 and other ligands, also contributes to the suppression of T cell activity, and is upregulated in chronic infections and tumors[9,10]. Observations that TIM-3 inhibition reinvigorates exhausted T cells suggest a critical role of TIM-3 in sustaining the exhausted state[11].

TIGIT binds CD155, a ligand shared with co-stimulatory receptor CD226, and transmits inhibitory signals that decrease T cell activation and cytokine production[12]. TIGIT expression frequently co-occurs with PD-1 expression, reflecting a coordinated network of inhibitory pathways that collectively reinforce T cell exhaustion[13]. The concurrent expression of multiple checkpoint molecules on exhausted CD8+ T cells suggests a complex regulatory mechanism that ensures sustained inhibition and immune evasion by tumors.

Persistent antigenic stimulation in the TME perpetuates the expression of these inhibitory receptors, and leads to hierarchical and progressive loss of T cell function[14]. Understanding the individual and collective roles of these key checkpoint molecules is essential for devising effective therapeutic strategies to reverse T cell exhaustion and enhance anti-tumor immunity.

Mechanisms regulating CD8+ T cell exhaustion by immune checkpoint molecules

Although immune checkpoint molecules play critical roles in regulating CD8+ T cell exhaustion, the intracellular phenomena triggered by the activation of inhibitory receptors are not fully understood. PD-1 is expressed predominantly in exhausted CD8+ T cells in the TME. Binding of PD-1 to its ligands, PD-L1 and PD-L2, leads to the recruitment of phosphatases, such as SHP-2, which dephosphorylate signaling molecules downstream of TCR and CD28, and ultimately inhibit T cell activation and proliferation[15,16]. This interaction diminishes cytokine production and cytolytic activity, and consequently contributes to functional impairment.

CTLA-4 competes with CD28 for binding to B7 molecules on APCs. By outcompeting CD28, CTLA-4 effectively decreases co-stimulatory signals for full T cell activation[17]. Furthermore, CTLA-4 recruits phosphatases, which further dampen signal transduction pathways critical for T cell

function[18]. The cumulative effect of CTLA-4 engagement is decreased proliferation and survival of $CD8^+$ T cells in the tumor milieu.

LAG-3 binds MHC class II molecules and transduces inhibitory signals that impair T cell expansion and cytokine release. LAG-3 blockade rejuvenates exhausted $CD8^+$ T cells, and restores their proliferative ability and effector function[19]. Similarly, TIM-3, after binding its ligands—such as galectin-9, phosphatidylserine, and CEACAM1—transmits inhibitory signals that suppress T cell responses[20-22]. The engagement of TIM-3 attenuates TCR signaling, decreases Th1 cytokine production, and consequently exacerbates T cell exhaustion.

Through its interaction with CD155 on APCs, TIGIT delivers inhibitory signals that compromise T cell activity. TIGIT competes with the co-stimulatory receptor CD226 for ligand binding, thus tipping the balance toward immune suppression[23]. The signaling cascades initiated by TIGIT contribute to decreased cytokine secretion and impaired cytotoxic responses, and further promote the exhausted phenotype.

Collectively, immune checkpoints coordinate interconnected inhibitory networks driving $CD8^+$ T cell dysfunction. Blocking these inhibitory pathways with ICIs reactivates exhausted T cells and restores anti-tumor immunity. Clinically, PD-1/CTLA-4 blockade has shown therapeutic potential against immune-resistant malignancies[24,25]. However, the downstream signaling mechanisms by which these immune checkpoint molecules induce $CD8^+$ T cell exhaustion are not yet fully understood. Although the mechanisms underlying the PD-1 pathway are relatively well understood, their variability across tumor types and patient populations remains a substantial issue. In addition, whether PD-1 acts synergistically with other inhibitory receptors requires further investigation. Co-expression of these receptors and their joint effects on T cell function, as well as combined therapeutic approaches to reverse this synergistic inhibition, are important directions for future research.

Effects of the TME on $CD8^+$ T cell dysfunction

TME conditions promote $CD8^+$ T cell exhaustion

The TME drives $CD8^+$ T cell exhaustion and significantly impedes their ability to elicit effective anti-tumor responses. Immunosuppressive regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs) facilitate an environment promoting T cell exhaustion. These cells secrete immunosuppressive cytokines, such as IL-10 and TGF-β, which hinder the proliferation and effector function of $CD8^+$ T cells[26,27].

Fibroblasts drive immunosuppression through cytokine/chemokine secretion and extracellular matrix (ECM) remodeling, thus critically shaping T cell infiltration and functionality[28]. The direct interactions between fibroblasts and $CD8^+$ T cells are mediated by immune checkpoint molecule expression. Notably, in response to pro-inflammatory cytokines or oncogenic stimuli, TME fibroblasts express high levels of PD-L1, which directly interacts with PD-1 on $CD8^+$ T cells, and subsequently inhibits their activation, clonal expansion, and cytotoxic potential. Immunohistochemistry has confirmed fibroblast PD-L1/T cell PD-1 co-localization in tumor regions[29]. Furthermore, the expression of other inhibitory ligands, such as B7-H4 and VISTA, on fibroblasts intensifies immunosuppression and exacerbates T cell exhaustion.[30,31]. Fibroblasts profoundly influence $CD8^+$ T cell function by altering local cytokine and chemokine levels. These cells secrete various factors, such as TGF-β, which fosters an immunosuppressive environment and induces T cell exhaustion[32]. Moreover, fibroblasts modify the ECM, creating physical barriers that can impede T cell motility and access to APCs, thereby exacerbating T cell exhaustion and immunological tolerance.

Hypoxia, a TME hallmark, further exacerbates T cell exhaustion. Hypoxic conditions within tumors stabilize hypoxia-inducible factor 1α, a transcription factor that reprograms T cell metabolism, ultimately driving the cells into a state of metabolic insufficiency.[33]. This metabolic stress impairs T cells' ability to sustain effector function, and promotes an exhausted phenotype characterized by upregulation of inhibitory receptors, such as PD-1 and CTLA-4[34,35].

Adenosine, another suppressive factor, is abundant in the TME. Tumor cells and associated stromal cells express ectonucleotidases, such as CD39 and CD73, which convert ATP to adenosine. Adenosine binding to the A2A receptor on T cells triggers signaling cascades that diminish the cells' cytotoxic activity and proliferative ability[36,37]. Consequently, the $CD8^+$ T cells have diminished ability to recognize and kill cancer cells.

In addition, the TME is rich in reactive oxygen species (ROS) and other oxidative stress-inducing agents that limit T cell function. ROS contribute to energy dysfunction by impairing

TCR signaling and downregulating the expression of important co-stimulatory molecules[38]. Oxidative stress induces the expression of additional inhibitory receptors and ligands, and consequently perpetuates the cycle of exhaustion[39].

The antigenic burden within the TME, characterized by the continued presence of tumor antigens, also plays a critical role in T cell exhaustion. Continual antigen exposure without adequate co-stimulation or assistance from other immune cells leads to progressive loss of T cell function[40]. This phenomenon is characterized by a hierarchical loss of cytokine production, diminished proliferative potential, and sustained expression of exhaustion markers.

The interplay between lactate accumulation (*via* tumor glycolysis) and histone deacetylase (HDAC) activation is a novel axis of TME-induced exhaustion. Lactate, a byproduct of Warburg metabolism, directly inhibits histone acetylation at effector gene loci and consequently silences effector gene expression[41]. Concurrently, targeting HIF-1α abrogates PD-L1-mediated immune evasion by suppressing PD-L1 expression on malignant and myeloid regulatory cells, and ultimately causes tumor rejection[42]. This dual metabolic-epigenetic suppression suggests that therapies combining LDHA inhibitors (to decrease lactate) with HDAC inhibitors (to restore acetylation) have potential to synergistically rejuvenate exhausted T cells.

Collectively, the TME orchestrates multifaceted, dynamic processes that drive CD8+ T cell exhaustion. Understanding these mechanisms is critical for developing therapeutic strategies that reverse T cell dysfunction and enhance anti-tumor immunity. The interplay among various cellular and molecular components within the TME creates a complex network that perpetuates T cell exhaustion, thus posing major challenges in effective cancer immunotherapy.

Strategies to reverse CD8+ T cell exhaustion in the TME

The TME comprises an intricate network of stromal cells, immunosuppressive entities, metabolic restrictions, and hypoxic conditions, which significantly impede effective anti-tumor immunity. Modulation of these components has been found to reinvigorate exhausted CD8+ T cells and to restore their cytotoxic potential against malignancies (**Figure 2**).

One promising approach involves depletion or reprogramming of immunosuppressive cells, such as Tregs, MDSCs, and TAMs. Tregs, which normally maintain immune homeostasis, are co-opted by tumors, thereby suppressing effector T cell function. Strategies targeting Treg-specific pathways, such as CTLA-4 or CD25, mitigate these suppressive effects and enhance CD8+ T cell activity[43]. Similarly, inhibition of MDSC recruitment and function through agents such as colony-stimulating factor 1 receptor (CSF1R) inhibitors or chemokine receptor blockers has been found to alleviate T cell suppression[44,45]. TAMs can be reprogrammed from the pro-tumoral M2 phenotype to the anti-tumor M1 state with agents such as CD40 agonists, thereby fostering an environment conducive to T cell activity[46].

Another strategy involves modulating the TME metabolic landscape. Tumors frequently create a metabolically hostile environment by consuming glucose and producing lactic acid, thereby impairing T cell function[47]. Enhancing nutrient availability by inhibiting cancer cell glycolysis[48] or providing alternative metabolic substrates[49] has been found to improve T cell function. Additionally, blocking the production of adenosine, a key immunosuppressive TME metabolite, or adenosine-mediated signaling further enhances CD8+ T cell responses[50].

Hypoxia-inducible factors induce the expression of immunosuppressive molecules and metabolic alterations that hinder T cell function[51]. Targeting these pathways with hypoxia-activated prodrugs or inhibitors of hypoxia-inducible factor signaling restores T cell efficacy[52]. Moreover, normalizing aberrant tumor vasculature with agents such as VEGF inhibitors has been found to improve oxygenation, subsequently increasing immune cell infiltration and function[53].

Adoptive T cell therapies, including treatments with CAR-T and TCR-T cells, have immense potential when combined with TME-modulating strategies. Engineering T cells to resist TME-induced exhaustion through the expression of dominant-negative receptors or metabolic reprogramming significantly enhances anti-tumor efficacy[54]. Furthermore, preconditioning the TME with radiation or chemotherapy to reduce immunosuppressive cell populations and enhance antigen presentation potentiates the effects of adoptively transferred T cells[55,56].

Emerging evidence indicates that the gut microbiome is a critical regulator of anti-tumor immunity and CD8+ T cell efficacy. *Bifidobacterium* and *Akkermansia muciniphila* have been shown to enhance the efficacy of ICIs by promoting T cell priming through microbial metabolites (e.g., short-chain fatty acids, SCFAs)[57,58]. SCFAs epigenetically reprogram exhausted T cells *via* HDAC inhibition, thereby reactivating effector gene

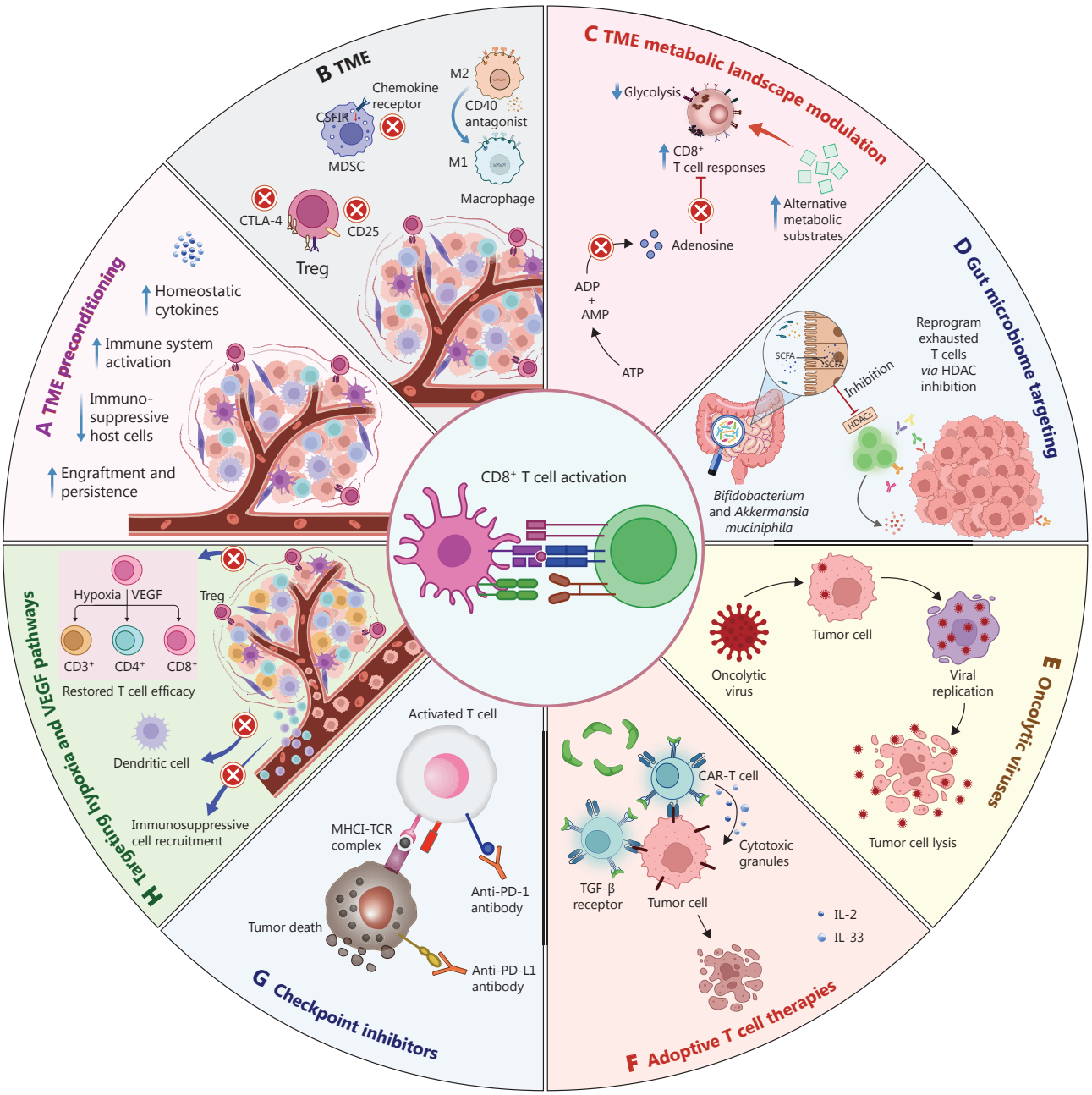


Figure 2 Strategies to revise CD8+ T cell exhaustion in the TME. (A) TME preconditioning. Preconditioning of the TME with radiation or chemotherapy to decrease immunosuppressive cell populations and enhance antigen presentation potentiates the effects of adoptively transferred T cells. (B) TME. TME cells can be targeted to prevent T cell exhaustion. CD8+ T cell activity can be enhanced through strategies targeting Treg-specific pathways such as CTLA-4 or CD25; inhibiting MDSC recruitment and function with agents such as CSF1R inhibitors or chemokine receptor blockers; or reprogramming TAMs from the pro-tumoral M2 phenotype to the anti-tumor M1 state with agents such as CD40 agonists. (C) TME metabolic landscape modulation. Metabolic reprogramming, such as shifting from oxidative phosphorylation to glycolysis in T cells, preventing ATP conversion to adenosine production, or targeting cancer cells through alternative metabolic substrates (e.g., inhibition of lactate dehydrogenase A to

decrease lactate production), further enhances CD8+ T cell responses. (D) Gut microbiome targeting. Gut microorganisms such as *Bifidobacterium* and *Akkermansia muciniphila* enhance ICI efficacy by promoting T cell priming through microbial metabolites. (E) Oncolytic viruses. Oncolytic viruses designed to selectively infect and lyse tumor cells, thus releasing tumor antigens and inducing local inflammation, provide another innovative approach for reshaping the TME. (F) Adoptive T cell therapies. CAR-T cells with dominant-negative TGF-β receptors and IL-2 or IL-33 secretion ability have shown promise in mitigating TME suppression. (G) Checkpoint inhibitors. Checkpoint inhibitors such as PD-1/PD-L1 blockers primarily neutralize immune checkpoint molecules on the surfaces of T cells, thus preventing them from binding ligands, and subsequently inhibiting T cell function or apoptosis. (H) Targeting hypoxia and VEGF pathways. VEGF inhibitors and inhibitors of hypoxia-inducible factor restore T cell efficacy. Normalizing aberrant tumor vasculature with agents such as VEGF inhibitors can improve oxygenation, subsequently increasing immune cell infiltration and function. ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CAR-T, chimeric antigen receptor T; CD, cluster of differentiation; CSF1R, colony-stimulating factor 1 receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HDACs, histone deacetylases; ICIs, immune checkpoint inhibitors; IL-2, interleukin-2; IL-33, interleukin-33; MDSCs, myeloid-derived suppressor cells; MHCI, major histocompatibility complex I; PD-1, cell death protein 1; PD-L1, programmed death-ligand 1; SCFAs, short-chain fatty acids; TAMs, tumor-associated macrophages; TCR, T cell receptor; TGF-β, transforming growth factor beta; TME, tumor microenvironment; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor.

networks[59]. In contrast, dysbiosis of the gut microbiota, characterized by an overabundance of pro-inflammatory species, may exacerbate T cell exhaustion *via* IL-17-driven inflammation or catabolism of tryptophan into immunosuppressive kynurenine[59]. Microbiome-targeting interventions (fecal microbiota transplantation, probiotics, or diet) show therapeutic potential against exhaustion. Further exploration of the microbiome-TME interplay will be crucial for developing precision immunotherapies against CD8+ T cell exhaustion.

Within the immunosuppressive TME, fibroblasts drive T cell exhaustion through the secretion of immunosuppressive cytokines (TGF-β and IL-10) and ECM proteins[60,61]. These cells establish peritumoral biophysical barriers that impede immune recognition, thus potentiating tumor progression and metastatic spread while compromising therapeutic efficacy. Detailed understanding of these mechanisms has provided critical insights into how the immunosuppressive environment can be altered to reactivate T cell function and improve patient outcomes. Notably, checkpoint inhibitors (PD-1/PD-L1 blockers) effectively disrupt fibroblast-mediated exhaustion mechanisms through neutralizing T cell inhibitory signals[29]. Furthermore, agents that degrade or inhibit the production of ECM components have been explored to potentially decrease the fibroblast-mediated barrier[62]. In addition, emerging approaches focus on fibroblast reprogramming toward pro-immunogenic phenotypes *via* genetic engineering or small-molecule modulation[63]. Future research should focus on exploring the specific mechanisms through which fibroblasts regulate T cell exhaustion *via* secreted factors and ECM alteration. Moreover, developing therapeutic strategies targeting fibroblasts, such as inhibiting PD-L1 or other immunosuppressive pathways, and interventions targeting ECM composition to enhance T cell infiltration and activity, will be key areas for future research. By understanding and strategically manipulating this interaction, the immune functions of CD8+ T cells can be enhanced. This approach promises more effective and sustained anti-tumor responses, and may open new avenues for cancer treatment and improving patient prognosis.

The use of oncolytic viruses designed to selectively infect and lyse tumor cells, thereby releasing tumor antigens and inducing local inflammation, is another innovative approach for reshaping the TME. These viruses can be engineered to express immunostimulatory molecules that further augment T cell responses[64]. Moreover, recent advancements in CAR-T cell engineering have demonstrated significant potential to overcome TME-induced exhaustion. For instance, "armored" CAR-T cells engineered to secrete cytokines such as IL-2 or IL-33 exhibit enhanced persistence and resistance to exhaustion by modulating the immunosuppressive microenvironment[65]. Furthermore, combinatorial approaches integrating CAR-T cells with dominant-negative TGF-β receptors have shown promise in mitigating TME suppression and preserving T cell effector function[66]. These innovations highlight the

potential of next-generation CAR-T therapies to act synergistically with checkpoint inhibitors and reshape the TME for sustained anti-tumor responses.

Although ICIs and adoptive T cell therapies can restore anti-tumor immunity, they frequently trigger immune-related adverse events, such as colitis, pneumonitis, and dermatitis, owing to systemic T cell activation targeting healthy tissues. For instance, anti-CTLA-4 therapies are associated with higher rates of severe immune-related adverse events than anti-PD-1 agents, thus underscoring the need for precision in targeting exhaustion pathways[67].

Despite the crucial role of the TME in driving CD8$^+$ T cell exhaustion, several key issues remain to be addressed. First, the mechanism underlying synergy among immunosuppressive cells (Tregs/MDSCs/TAMs) in driving exhaustion, particularly their cytokine-mediated (IL-10/TGF-β) T cell regulation, must be elucidated. Second, hypoxia/adenosine/ROS affect T cell bioenergetics, and their crosstalk with immunosuppressive networks requires systematic analysis. Third, antigen-driven T cell dysfunction reversal strategies through antigen-presentation machinery and co-stimulation modulation must be prioritized. Fourth, stromal-mediated T cell exclusion mechanisms require mechanistic exploration to inform barrier-disrupting therapies. Pharmacological innovations should target immunosuppressive cell depletion, TME metabolic reprogramming, and hypoxia/oxidative stress alleviation to potentiate combination immunotherapies. Concurrently, gene-edited T cells with microenvironment resistance and TME-remodeling modalities (tumor vaccines/oncolytic viruses) are promising translational frontiers. Multipronged TME modulation strategies are expected to have transformative potential in reversing CD8+ T cell exhaustion and optimizing immunotherapeutic efficacy.

CD8$^+$ T cell exhaustion is regulated by metabolic reprogramming

Metabolic pathways in CD8$^+$ T cell exhaustion

Exhausted CD8$^+$ T cells in the TME show significantly altered metabolism and consequent functional effects. A shift from oxidative phosphorylation to glycolysis, marked by increased glucose dependence and decreased mitochondrial respiration, is a prominent change mirroring the Warburg effect observed in cancer cells. Glycolytic reprogramming results in intracellular accumulation of lactate, which in turn contributes to the immunosuppressive environment[68]. For instance, lactate accumulation in the TME inhibits histone acetylation, whereas mitochondrial dysfunction decreases acetyl-CoA availability, both of which suppress the expression of effector genes in CD8$^+$ T cells[69].

Exhausted CD8$^+$ T cells manifest severe mitochondrial dysfunction marked by biogenesis suppression, mass depletion, and membrane depolarization. Mitochondrial dysfunction diminishes fatty acid oxidation (FAO), an essential pathway for sustaining long-term cellular energy needs[70]. FAO impairment disrupts the balance between NAD$^+$/NADH and ATP production, and further contributes to cellular exhaustion[71].

Another significant alteration in exhausted CD8$^+$ T cells is the disruption of amino acid metabolism. The availability of key amino acids, such as glutamine, arginine, and tryptophan, is limited inside the TME[72]. Decreased uptake and utilization of these amino acids hinders the synthesis of nucleotides and polyamines critical for T cell function[73,74]. In addition, tryptophan catabolism by indoleamine 2,3-dioxygenase in the TME generates kynurenine[75], a compound that has immunosuppressive effects and contributes to T cell exhaustion[76].

Lipid metabolism is also significantly affected. Exhausted CD8$^+$ T cells show altered lipid uptake and storage[77]. Aberrant lipid metabolism contributes to ROS generation, promotes oxidative stress, and impairs cellular functions[78]. Dysregulated cholesterol metabolism, including decreased efflux and increased esterification of cholesterol, disrupts membrane fluidity and signaling pathways critical for T cell function[79].

The mammalian target of rapamycin (mTOR) and adenosine monophosphate-activated protein kinase (AMPK) signaling pathways are critically involved in CD8$^+$ T cell exhaustion. Hyperactivation of the mTOR pathway promotes anabolic metabolism, including glycolysis and synthesis, at the expense of catabolic processes, such as FAO[80,81]. In contrast, impaired activation of AMPK, an energy sensor that promotes catabolic pathways, decreases FAO[82].

Despite extensive studies on metabolic reprogramming in CD8$^+$ T cell exhaustion, several key issues remain to be addressed. First, the precise mechanisms through which enhanced glycolysis and decreased mitochondrial function affect T cell energy metabolism and signaling, and how these changes relate to other immunosuppressive mechanisms, require further investigation. Second, the effects of altered amino acid and lipid metabolism on T cell proliferation, differentiation, and effector

function must be determined, and strategies to restore T cell function by modulating these metabolic pathways must be developed. Moreover, further investigation is necessary regarding the interplay between metabolic reprogramming and epigenetic modifications, their influence on gene expression and function in T cells, and strategies to alter the epigenetic state of T cells by modulating metabolic pathways. Competition for nutrients and metabolic stress within the TME might exacerbate T cell exhaustion. Future studies must develop strategies to enhance T cell function by improving TME metabolic conditions, such as pharmacological interventions to inhibit glycolysis, enhance mitochondrial function, improve amino acid and lipid metabolism, and enhance T cell anti-tumor activity through combined metabolic regulators. Moreover, leveraging gene editing and cell engineering technologies to enhance T cell resistance to metabolic stress through tumor vaccines and oncolytic viruses is an important direction for future research. Understanding the intricate relationship between metabolism and CD8+ T cell exhaustion is crucial for developing therapeutic strategies aimed at rejuvenating exhausted T cells and enhancing their anti-tumor responses. These metabolic alterations not only impair energy production but also intersect with transcriptional and epigenetic networks.

Modulation of CD8+ T cell exhaustion by transcription factors and non-coding RNAs

Transcription factors and CD8+ T cell exhaustion

TCF-1 and LEF-1 are critical transcription factors that regulate CD8+ T cell exhaustion by maintaining their stem cell-like characteristics. These factors interact with β-catenin in the Wnt signaling pathway, and subsequently activate downstream target genes, and promote self-renewal and pluripotency. However, the preservation of stem-like characteristics restricts differentiation into mature effector T cells and suppresses effector function, such as cytotoxicity and cytokine production[83,84].

Members of the thymocyte selection-associated high-mobility group box (TOX) family are highly expressed in exhausted CD8+ T cells, in which they interact with chromatin remodeling complexes and subsequently influence gene expression. TOX plays a crucial role in immune regulation by modulating the expression of inhibitory receptors (such as PD-1 and LAG-3) and suppressing the activation of genes associated with effector T cell function[85]. High TOX expression is closely associated with T cell exhaustion, and TOX is a key molecule involved in maintaining the exhausted phenotype.

Members of the nuclear receptor subfamily 4A (NR4A) family, including NR4A1, NR4A2, and NR4A3, also play major roles in CD8+ T cell exhaustion. These transcription factors regulate gene expression by directly binding specific promoters. NR4A family members typically promote a quiescent state and inhibit effector T cell function, and are involved in maintaining an exhausted T cell state[86].

B-lymphocyte-induced maturation protein 1 (Blimp-1) is a key transcriptional repressor with a major role in CD8+ T cell exhaustion. Blimp-1 inhibits the expression of genes associated with effector T cells, such as T-bet and Eomes[87,88]. In addition, Blimp-1 regulates genes associated with the cell cycle and apoptosis, and ultimately affects the survival and proliferation of exhausted T cells.

Basic leucine zipper transcription factor (BATF) also plays a major role in CD8+ T cell exhaustion. BATF forms heterodimers with AP-1 family members, such as JUN and FOS, thereby regulating gene expression[89]. BATF maintains T cell exhaustion by activating the expression of genes encoding inhibitory receptors and cell cycle regulators[90].

The intricate interplay among transcription factors, such as TCF-1, LEF-1, TOX, NR4A, Blimp-1, and BATF, orchestrates the phenotypic and functional exhaustion of CD8+ T cells, and critically affects immune surveillance and response dynamics within the TME. These transcriptional regulators sustain the stem-like plasticity of exhausted T cells and modulate the expression profiles of inhibitory receptors, thus attenuating their cytotoxic potential and cytokine secretion capabilities. A thorough understanding of the molecular mechanisms is imperative for devising innovative strategies to invigorate T cell functionality within the tumor milieu. Strategic targeting of these transcription factors or their downstream signaling cascades might rejuvenate the exhausted T cell pool and augment their anti-tumor efficacy. This approach has potential to significantly enhance the outcomes of immunotherapeutic interventions such as topoisomerase II inhibitors, which are designed to reinvigorate the immune responses by antagonizing inhibitory signals.

Non-coding RNAs and CD8+ T cell exhaustion

miRNAs finely tune the immune response of CD8+ T cells by targeting key signaling molecules and transcription factors.

For example, miR-150 influences the immune response by downregulating cellular activation and effector function[91]. These miRNAs decrease the proliferation and production of effector molecules by inhibiting key components of signaling pathways, such as MYB and CCND1[92,93]. The regulatory effects of miRNAs are crucial for maintaining exhausted T cells in a stable state.

LncRNAs such as HOTAIR and MALAT1 regulate the expression of exhaustion-associated genes by affecting chromatin structure and transcriptional activity. These lncRNAs influence gene expression patterns by interacting with transcription factors, RNA-binding proteins, or chromatin remodeling factors. For instance, HOTAIR recruits the PRC2 complex, which in turn promotes epigenetic silencing of genes[94], whereas MALAT1 regulates gene splicing patterns by affecting the localization of splicing factors, such as SR proteins[95].

CircRNAs, a class of circular RNA molecules, also have potential regulatory roles in CD8$^+$ T cell exhaustion. CircRNAs influence post-transcriptional regulatory networks by sequestering miRNAs or interacting with RNA-binding proteins[96]. Although the specific mechanisms through which circRNAs act on exhausted T cells are not fully understood, these molecules might participate in regulating the exhausted state by affecting miRNA activity and protein stability.

Competitive endogenous RNA (ceRNA) networks regulate gene expression by sharing miRNA response elements. During CD8$^+$ T cell exhaustion, ceRNA networks have been suggested to influence the expression of key transcription factors and signaling molecules by competitively binding miRNAs[97]. Complex interactions within this network might significantly affect the function and stability of exhausted T cells.

The regulatory landscape of ncRNAs, including miRNAs, lncRNAs, circRNAs, and the ceRNA network, along with RNA-binding proteins (RBPs), plays critical roles in modulating CD8$^+$ T cell exhaustion, particularly within tumors. These RNA species and RBPs fine-tune the immune response by orchestrating intricate networks of gene expression, RNA stability, and protein synthesis. Their involvement in promoting T cell exhaustion underscores the potential for targeting these molecules to reinvigorate T cell function within tumors. Several key issues remain unaddressed. First, the precise mechanisms through which transcription factors such as TCF-1, LEF-1, TOX, NR4A, Blimp-1, and BATF synergistically maintain T cell exhaustion, and how these factors interact with other immunosuppressive mechanisms, must be further explored. Second, how non-coding RNAs, including miRNAs, lncRNAs, circRNAs, and RBPs, regulate gene expression, RNA stability, and protein synthesis in T cells must be clarified. In addition, how the interplay between transcription factors and ncRNAs influences T cell gene expression and function, and whether the epigenetic state of T cells is altered by these interactions, requires further investigation. Future studies must crucially attempt to restore CD8$^+$ T cell function by modulating the levels and activity of exhaustion-associated transcription factors and non-coding RNAs. These insights would not only underscore the potential of RNA-centric therapeutic strategies as adjuncts to conventional immunotherapies but also potentially pave the way to novel combinatorial approaches that significantly enhance therapeutic efficacy against cancer.

Epigenetic regulation of CD8$^+$ T cell exhaustion

Epigenetic modifications associated with CD8$^+$ T cell exhaustion

Epigenetic modifications play crucial roles in the regulation of CD8$^+$ T cell exhaustion. An important aspect of this regulation is methylation of cytosine residues in CpG dinucleotides, a modification that typically leads to gene silencing. In exhausted CD8$^+$ T cells, specific DNA methylation patterns emerge and contribute to the repression of genes essential for effector function, while promoting the expression of inhibitory receptors, such as PD-1, CTLA-4, and TIM-3[98,99]. This methylation landscape reinforces the exhausted phenotype, and renders CD8$^+$ T cells less responsive to antigen stimulation and less capable of producing cytokines, such as IFN-γ and TNF-α[100,101]. The persistence of these methylation marks, even after antigen removal, suggests that they constitute epigenetic memories that maintain an exhausted state.

Beyond DNA methylation, CD8$^+$ T cell exhaustion is also modulated by histone modifications. Histone acetylation is typically associated with transcriptional activation and is markedly diminished at loci encoding effector molecules in exhausted T cells. In contrast, histone deacetylation at these sites is facilitated by HDACs, which contribute to chromatin condensation and gene repression[102]. Furthermore, histone methylation patterns in exhausted CD8$^+$ T cells significantly differ from those in their functional counterparts. For example, trimethylated lysine 27 of histone H3 (H3K27me3), a

repressive mark, is enriched in the promoters of genes critical for effector function, thus limiting their transcription. In contrast, diminished permissive marks, such as H3K4me3, at these loci further inhibit gene expression[103].

Another layer of complexity is the interplay among epigenetic modifications. For example, DNA methylation influences histone modification patterns and vice versa. Methyl-CpG-binding domain proteins recruit HDACs and histone methyltransferases to methylated DNA regions, thus reinforcing the repressive chromatin state[104]. These interactions highlight the coordinated nature of epigenetic regulation in the establishment and maintenance of exhausted phenotypes.

Emerging evidence indicates the involvement of ncRNAs in the epigenetic regulation of CD8+ T cell exhaustion. LncRNAs and miRNAs modulate the expression of genes associated with exhaustion by interacting with chromatin-modifying enzymes, and subsequently influencing DNA methylation and histone modification landscapes. For example, specific lncRNAs have been shown to recruit histone-modifying complexes to target gene loci, thereby altering their transcriptional activity in exhausted T cells[105].

Understanding of the epigenetic landscape associated with CD8+ T cell exhaustion provides valuable insights into the mechanisms underlying the dysfunctional state and opens potential therapeutic avenues to the reversal of exhaustion by targeting epigenetic modifications. Agents such as DNA methyltransferase inhibitors and HDAC inhibitors are currently being tested for their ability to rejuvenate exhausted T cells, restore effector function, and enhance their anti-tumor activity[106]. These strategies have promise in enhancing the efficacy of immunotherapies and improving the clinical outcomes of patients with cancer.

Effects of epigenetic changes on the transcriptional programs of exhausted CD8+ T cells

Epigenetic modifications involved in transcriptional reprogramming of exhausted CD8+ T cells profoundly influence their functional state and response to immunotherapy. These modifications include DNA methylation, histone modifications, and chromatin remodeling, which contribute to the establishment and maintenance of an exhausted phenotype.

Chromatin remodeling complexes, including SWI/SNF and NuRD, dynamically regulate nucleosome positioning and chromatin structure, and consequently influence gene expression. In exhausted CD8+ T cells, the activity of these complexes is often skewed toward maintaining a closed chromatin configuration, which prevents the transcription of genes necessary for T cell activation and effector function. Integrating signals from the TME further exacerbates these epigenetic alterations and results in a feedback loop that sustains the exhausted state[107]. Understanding of the interplay between these chromatin remodeling factors and CD8+ T cell transcriptional programs is essential for developing strategies to rejuvenate exhausted cells.

Several issues regarding epigenetic regulation in CD8+ T cell exhaustion remain to be addressed. First, the exact mechanisms through which DNA methylation and histone modifications synergistically maintain the exhausted state of T cells must be determined. How these modifications interact with other immunosuppressive mechanisms is also unclear. Second, the roles of ncRNAs in modulating chromatin-modifying enzymes that influence T cell function, and strategies to restore T cell function by modulating ncRNAs, are key points for future studies. In addition, how the interplay between epigenetic modifications and transcription factors regulates gene expression and function in T cells must be elucidated, and strategies to modify the epigenetic state of T cells through these interactions should be developed.

Epigenetic reprogramming of CD8+ T cells has emerged as a therapeutic imperative for functional recovery. CRISPR-dCas9-based tools offer promising avenues for direct modulation of epigenetic marks and restoration of the functional competence of exhausted CD8+ T cells. Furthermore, combining epigenetic therapies with existing checkpoint inhibitors has potential to synergistically improve therapeutic outcomes by removing inhibitory signals and promoting the reactivation of exhausted T cells. As understanding of the epigenetic regulation of CD8+ T cell exhaustion deepens, the potential to develop innovative and effective immunotherapeutic interventions will continue to expand, offering new hope for patients with cancer.

Exhaustion of other populations of immune cells

Beyond CD8+ T cells, several other cellular constituents of the immune system are susceptible to exhaustion. Several key immune cell types and the etiologies underlying their exhaustion are described below, and their biological significance is discussed (**Figure 3**).

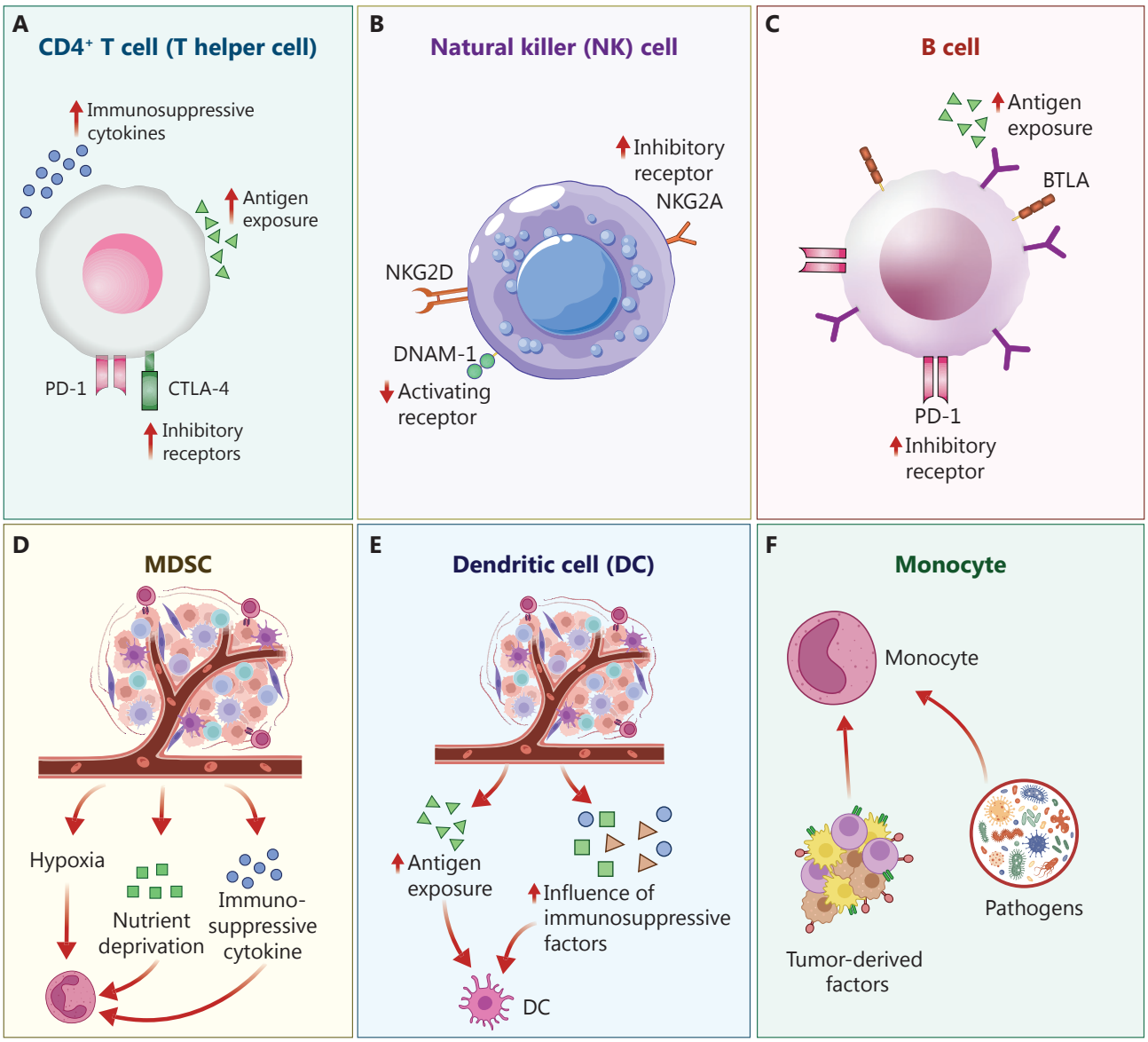


Figure 3 Exhaustion of other populations of immune cells. (A) CD4+ T cell exhaustion. CD4+ T cell exhaustion occurs because of upregulation of inhibitory receptors, prolonged antigen exposure, and the presence of immunosuppressive cytokines. (B) NK cell exhaustion. NK cell exhaustion is triggered by the persistent engagement of the checkpoint NKG2A and killer cell immunoglobulin-like receptors and diminished signaling through the activating receptors (e.g., NKG2D and DNAM-1). (C) B cell exhaustion. B cell exhaustion occurs primarily because of upregulation of inhibitory receptors (such as PD-1 and BTLA) and prolonged antigen exposure. (D) MDSC exhaustion. MDSC exhaustion occurs because of hypoxia, nutrient deprivation, and the presence of immunosuppressive cytokines in the TME. (E) Dendritic cell (DC) exhaustion. DCs are exhausted in the TME because of antigen exposure and the influence of immunosuppressive factors. (F) Monocyte exhaustion. Monocytes are exhausted in the presence of pathogens or various tumor-derived factors. BTLA, B and T lymphocyte attenuator; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DCs, dendritic cells; DNAM-1, DNAX accessory molecule 1; MDSCs, myeloid-derived suppressor cells; NK, natural killer; NKG2A, natural-killer group 2, member A; NKG2D, natural-killer group 2, member D; PD-1, cell death protein 1; TME, tumor microenvironment.

CD4+ T cells (T helper cells)

CD4+ T cells, particularly those with T helper 1 (Th1) or T helper 17 (Th17) phenotypes, may succumb to exhaustion during chronic infections or malignancies[108]. This phenomenon is influenced primarily by sustained antigenic exposure, expression of inhibitory receptors (e.g., PD-1 and CTLA-4), and the presence of immunosuppressive cytokines (such as IL-10 and TGF-β). These factors decrease cytokine production and support for B cells and CD8+ T cells, thus affecting the overall immune response[109]. CD4+ T cell exhaustion dampens immune responses, and consequently prevents excessive inflammation and tissue damage. However, it also hampers effective immune surveillance and the clearance of persistent pathogens and tumors.

Natural killer (NK) cells

NK cells, critical orchestrators of the innate immune response, are integral to the body's defense against tumors and virally infected cells. In chronic viral infections and cancer, NK cell exhaustion is triggered by the persistent engagement of the checkpoint NKG2A and killer cell immunoglobulin-like receptors, and diminished signaling through the activating receptors (e.g., NKG2D and DNAM-1)[110-113]. The resultant decreased cytotoxicity and impaired cytokine production compromise innate immune surveillance. NK cell exhaustion regulates the intensity of innate immune responses, thereby preventing uncontrolled cytotoxicity and inflammation; however, it also decreases the ability of the body to combat early-stage tumors and viral infections.

B cells

B cell exhaustion during chronic infections and malignancies is marked by a decline in antibody production and compromised class switching. This condition is frequently driven by chronic antigen exposure and upregulation of inhibitory receptors (such as PD-1 and BTLA)[114]. The exhaustion of B cells diminishes the humoral immune response, and subsequently affects long-term protection against pathogens and tumor antigens. B cell exhaustion modulates the humoral immune response and prevents the overproduction of potentially harmful antibodies, but simultaneously limits effective long-term immunity and memory responses.

MDSCs

MDSCs are a heterogeneous population of myeloid cells that accumulate in pathological conditions, such as cancer and chronic infections. MDSCs can become functionally exhausted, lose their ability to differentiate into mature immune cells, and promote immunosuppression. This exhaustion is influenced by the TME, which encompasses factors such as hypoxia, nutrient deprivation, and immunosuppressive cytokines. MDSC exhaustion contributes to the maintenance of immune homeostasis by suppressing excessive inflammatory responses, but also facilitates the progression of tumors and chronic infections by inhibiting effective immune surveillance and response.

Dendritic cells (DCs)

DCs are APCs crucial for the initiation and modulation of immune responses. In chronic infections and cancer, DCs can become exhausted. This exhaustion is characterized by decreased antigen presentation, impaired co-stimulatory molecule expression, and altered cytokine secretion, and it is driven primarily by persistent antigen exposure and the influence of immunosuppressive factors within the TME[115]. DC exhaustion helps prevent the overactivation of T cells and consequent tissue damage; however, it also impairs the initiation of adaptive immune responses, thus leading to ineffective clearance of pathogens and tumor cells.

Monocytes

Monocyte exhaustion, a critical adaptive response within the immune system, is often observed in chronic infections and malignancies. This phenomenon is characterized by a significant decrease in the functional capabilities of monocytes, including compromised antigen presentation, attenuated phagocytic activity, and diminished production of pro-inflammatory cytokines[116]. The complex and multifactorial etiology of monocyte exhaustion is driven predominantly by chronic exposure to pathogens or tumor-derived factors that create an immunosuppressive microenvironment. Monocyte exhaustion has several biological implications. In infectious diseases, monocyte exhaustion leads to inadequate microbial clearance and prolonged disease courses, and often increases morbidity and chronicity. In cancer, exhausted monocytes contribute

to the establishment and maintenance of a suppressive TME, which not only protects tumor cells against immune-mediated destruction, but also promotes tumor progression and metastasis. Targeting monocyte exhaustion is a promising approach for enhancing the efficacy of existing treatments. Strategies to rejuvenate exhausted monocytes, such as blocking inhibitory receptors or modulating their signaling pathways, might restore their function, and consequently improve outcomes in patients with chronic infections or cancer. Understanding and manipulating pathways that lead to monocyte exhaustion might play critical roles in next-generation immunotherapeutic strategies.

Understanding immune cell exhaustion is essential for the advancement of therapeutic interventions designed to bolster immune function in chronic infections and cancer. Revealing the molecular and cellular pathways that culminate in exhaustion would enable the formulation of strategies to reverse or preempt this debilitating state, and to revitalize the immune response. Immune cell exhaustion negatively affects not only these cells' own functions but also the entire immune response, thereby influencing disease treatment efficacy. In-depth investigation of the precise molecular mechanisms underlying the exhaustion of these immune cells is necessary, with a focus on the expression and signaling pathways of inhibitory receptors. Deciphering the mechanisms of exhaustion would support the development of innovative therapeutic strategies to rejuvenate these cells, potentially augment the efficacy of immunotherapy, and significantly improve patient outcomes. Subsequently, the development of novel biomarkers should be pursued to track and assess exhaustion levels of these cells, and facilitate more informed treatment strategies. Furthermore, innovative therapeutic methods should be pursued, including the development of antibodies or small-molecule inhibitors that target inhibitory receptors, as well as gene editing techniques aimed at reinvigorating cellular functions. Finally, the safety and efficacy of these therapeutic interventions must be rigorously evaluated in clinical trials to determine their applicability in managing cancer and persistent infections. Reinvigorating immune cells is a promising frontier for enhancing the outcomes of therapies for cancer and persistent infections.

Discussion

$CD8^+$ T cell exhaustion occurs in many tumors, and comprehensive understanding of its causes is critical. Herein, we summarized relevant factors and molecular mechanisms underlying $CD8^+$ T cell exhaustion (**Figure 4**). Clinical and experimental studies have shown that reinvigorating exhausted $CD8^+$ T cells can halt tumor progression. Reactivation of exhausted T cells may inadvertently expand self-reactive T cell clones that escaped immune surveillance, particularly in patients with pre-existing autoimmune conditions. Furthermore, epigenetic reprogramming agents can erase tolerance mechanisms, thus exacerbating autoinflammatory responses. Despite extensive studies, current understanding of the biological processes involved in $CD8^+$ T cells activation, the gene networks, and the molecular mechanisms underlying $CD8^+$ T cell exhaustion remains in nascent stages. Assessing $CD8^+$ T cell exhaustion would enable the precision of therapeutic interventions to be enhanced and facilitate the stratification of patients for personalized treatment strategies.

To reinvigorate exhausted $CD8^+$ T cells, a delicate balance must be maintained between reactivating these cells to attack cancer cells and preventing them from affecting normal tissues. Disruption of the immune system can lead to unintended and potentially severe autoimmune responses. Therefore, a deep understanding of the signal cascades involved in $CD8^+$ T cell exhaustion and reactivation is essential to mitigate harmful conditions. We expect that the use of checkpoint inhibitors together with other therapies—such as metabolic reprogramming agents, cytokine therapy, or adoptive cell transfer—could synergistically enhance anti-tumor responses, while preventing or overcoming resistance. Notably, advancements in CAR-T cell engineering, such as metabolic reprogramming and armored cytokine secretion, alongside CRISPR-based epigenetic editing of exhaustion-associated genes, have demonstrated unprecedented efficacy in preclinical models[117,118]. These approaches, when combined with immune checkpoint blockade, have potential to overcome resistance and enhance durable anti-tumor responses[56]. Emerging clinical frameworks leverage exhaustion-associated biomarkers to rationally design combination therapies. For example, patients with high CD73 tumors significantly benefit from anti-PD-1 combined with CD73 inhibitors[119]. Similarly, NR4A1/2 transcriptional activity correlates with resistance to immunology checkpoint blockades but sensitizes tumors to combination treatment with NR4A antagonists and a small molecule inhibitor[120]. These advances underscore the necessity of profiling exhaustion pathways to optimize combinatorial strategies. Despite substantial progress, the following key questions remain: (1) Can epigenetic "reset" of exhausted T cells achieve durable reinvigoration without promoting autoimmunity? (2) How

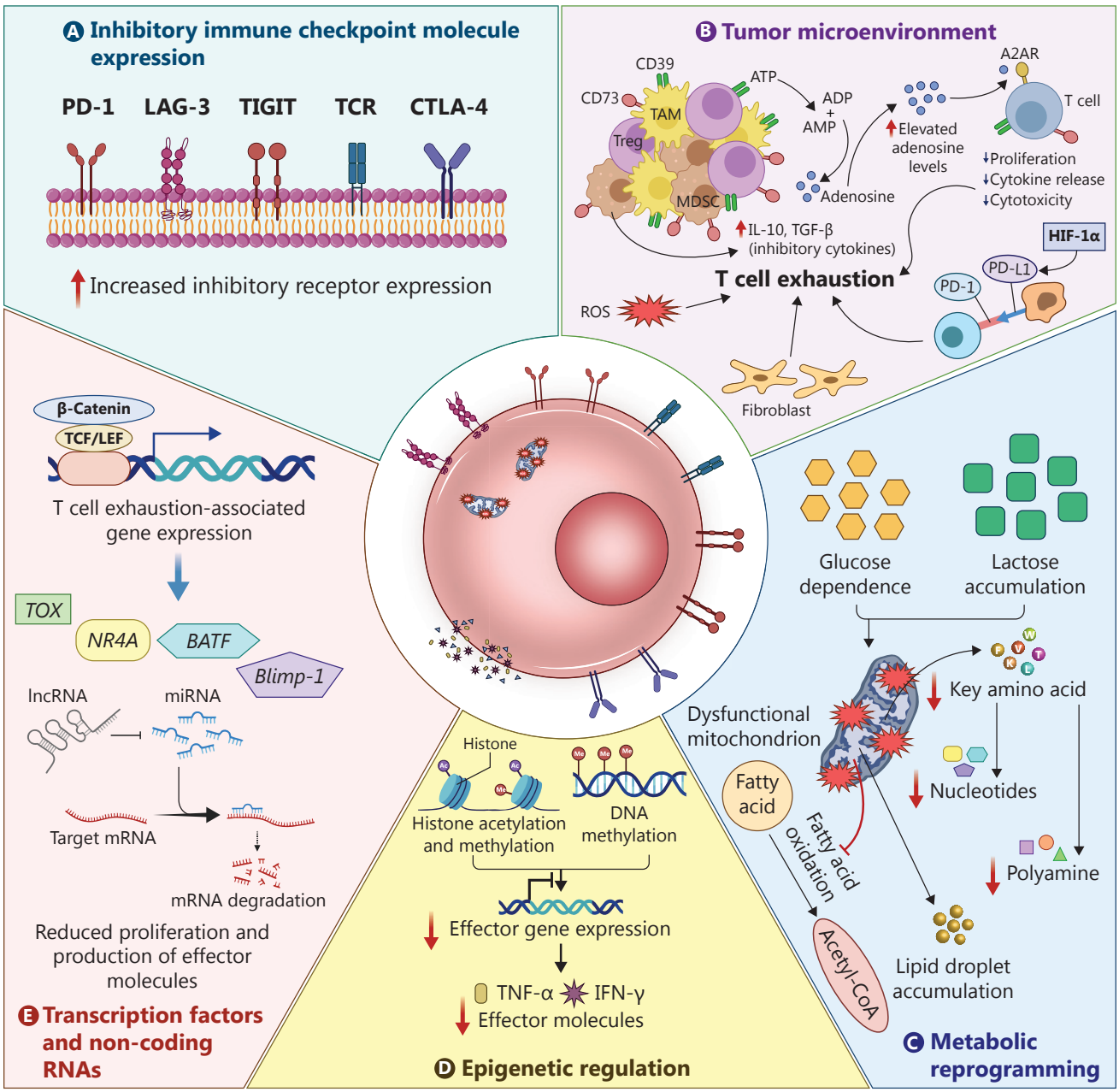


Figure 4 Determinants of CD8+ T cell exhaustion in cancer. (A) Inhibitory immune checkpoint molecules. The expression of inhibitory immune checkpoint molecules (PD-1, CTLA-4, LAG-3, TCR, and TIGIT) acts as a brake on T cell activity, thus restricting their ability to mount effective anti-tumor immune responses. (B) Tumor microenvironment (TME). The TME is rich in regulatory cells (Tregs, MDSCs, and TAMs), with elevated production of inhibitory cytokines (IL-10 and TGF-β), thereby restricting CD8+ T cell infiltration and function. Tumor cells and associated stromal cells express ectonucleotidases, such as CD39 and CD73, which convert ATP to adenosine. Elevated adenosine binds A2A receptors on T cells and subsequently decreases cytotoxic activity. ROS production further impairs TCR signaling, and upregulates inhibitory receptors and ligands, thus perpetuating the cycle of CD8+ T cell exhaustion. In addition, HIF-1α promotes PD-L1-mediated immune evasion and ultimately CD8+ T cell exhaustion. Moreover, stromal elements such as fibroblasts contribute to a physical and biochemical barrier, thereby modulating immune cell infiltration and activity. (C) Metabolic reprogramming. Glucose dependence and lactate accumulation decrease mitochondrial mass and exacerbate CD8+ T cell exhaustion. Mitochondrial dysfunction decreases FAO and consequently disrupts the balance between NAD+/NADH and ATP production, thus further aggravating CD8+ T cell exhaustion. Insufficiency in key amino acids

impairs the nucleotide and polyamine synthesis essential for $CD8^+$ T cell function. Lipid metabolism is altered, and increased lipid droplet accumulation contributes to ROS generation and oxidative stress. (D) Epigenetic regulation. DNA methylation leads to silencing of functional effector genes and downregulation of inhibitory receptors. Histone methylation and acetylation alter transcriptional activity, thereby decreasing effector molecule expression and maintaining the exhausted state. Collectively, these mechanisms underlie the complex crosstalk characterizing the exhaustion phenotype, and profoundly influence the persistence, functionality, and survival of $CD8^+$ T cells within the unfavorable and suppressive tumor milieu. (E) Transcription factors and non-coding RNAs. Transcription factors (such as TCF-1 and LEF-1) interact with β-catenin, thereby activating downstream target genes and maintaining the $CD8^+$ T cell exhaustion state through their stem cell-like characteristics. TOX, NR4A, Blimp-1, and BATF expression orchestrates the phenotypic and functional exhaustion of $CD8^+$ T cells by maintaining an exhausted state. Non-coding RNAs (lncRNAs, circRNAs, and ceRNAs) fine-tune $CD8^+$ T cell exhaustion-associated gene networks. ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; A2AR, A2A receptor; BATF, basic leucine zipper transcription factor; Blimp-1, B-lymphocyte-induced maturation protein 1; CD39, ectonucleoside triphosphate diphosphohydrolase-1; CD73, ecto-5′-nucleotidase; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ECM, extracellular matrix; FAO, fatty acid oxidation; HIF-1α, hypoxia-inducible factor-1 alpha; IFN-γ, interferon-gamma; IL-10, interleukin-10; LAG-3, lymphocyte activation gene 3; LEF-1, lymphoid enhancer-binding factor 1; MDSCs, myeloid-derived suppressor cells; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide, reduced; NR4A, nuclear receptor subfamily 4A; PD-1, cell death protein 1; PD-L1, programmed cell death-ligand 1; ROS, reactive oxygen species; TAMs, tumor-associated macrophages; TCF-1, T cell factor 1; TCR, T cell receptor; TGF-β, transforming growth factor beta; TIGIT, T cell immunoreceptors with Ig and ITIM domains; TME, tumor microenvironment; TNF-α, tumor necrosis factor alpha; TOX, members of the thymocyte selection-associated high-mobility group box; Tregs, regulatory T cells.

do fibroblast-derived exosomes deliver microRNAs to T cells, and can this delivery be blocked? (3) Can spatial multi-omics technologies resolve heterogeneity within exhausted T cell niches? Answering these questions will require interdisciplinary collaboration merging immunology, synthetic biology, and computational modeling. Clinically, biomarker-driven trials stratifying patients by exhaustion subtype might optimize checkpoint inhibitor efficacy, while avoiding futile treatment in "immune-desert" tumors.

Because $CD8^+$ T cell exhaustion is a complex process, interdisciplinary approaches are crucial for developing innovative solutions to $CD8^+$ T cell exhaustion. Understanding the complexity of $CD8^+$ T cell exhaustion is essential for tumor biology. This review highlighted the molecular mechanisms promoting $CD8^+$ T cell exhaustion. Approaches to reverse $CD8^+$ T cell exhaustion are expected to be promising in cancer treatment. In contrast to earlier reviews, our work advances 3 key perspectives: (1) the crosstalk between metabolic stress and epigenetic silencing of effector genes is a novel axis for intervention; (2) fibroblast-T cell interactions, which are often overlooked in discussions of the TME, are critically associated with checkpoint ligand expression and immune evasion; and (3) emerging technologies such as spatial multi-omics and transient epigenetic editing offer unprecedented precision in targeting exhaustion. Nevertheless, our analysis is limited by the heterogeneity in exhaustion markers across studies, thereby complicating direct mechanistic comparisons. Future efforts should prioritize standardized exhaustion biomarkers and subtype-specific therapeutic profiling.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Tao Zhong, Shuo Sun, Huabao Xiong.
Collected the data: Tao Zhong, Mingsheng Zhao, Bin Zhang.
Contributed data or analysis tools: Shuo Sun, Bin Zhang.
Performed the analysis: Tao Zhong, Shuo Sun.
Wrote the paper: Tao Zhong, Huabao Xiong.

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